CeA CRF Ephys Paper
STATISTICS REFERENCE:

ALL GRAPHPAD PRISM RESULTS FOR EVERY FIGURE

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Updated: 09/03/18

GENERAL NOTES

09/03/18:

Updated this document with the latest version of the figure legends and figures from Sonia.

Added figure 2B's statistics from old place in fig 4.

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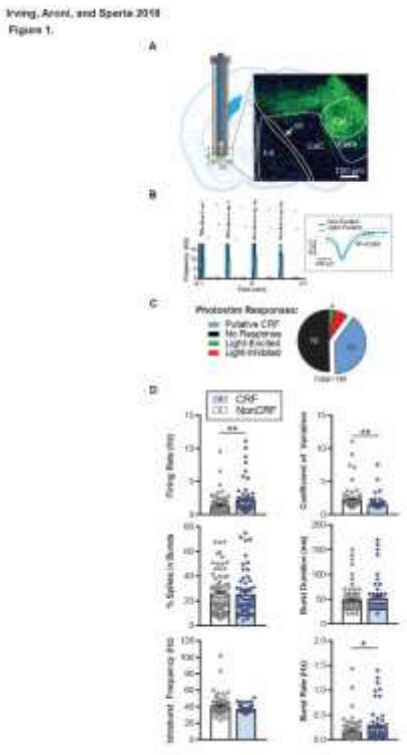
I. FIGURE 1

JPG



LEGEND

Figure 1. Optical identification of CRF neurons *in vivo.* **A)** Scaled diagram of optical-fiber coupled microarray implanted into the central amygdala of CRF-cre mice, and local CeA injection of AAV-DIO –Channelrhodopsin2-EYFP virus, whose expression is showed in the image. **B)** Peristimulus time histogram and scatter plot showing identified CRF neurons. Units were classified as CRF only if they fired within 10 ms of the onset of a 4 ms-long light pulse, and the light-evoked waveforms had an R^2 >0.9 compared to non-light evoked waveforms. **C)** The pie chart shows that out of 149 total units, 59 were identified as putative CRF neurons, 75 non-responsive to light, and a small population excited (4) or inhibited (11). Due to the low N for these light responses, we focused on CRF units vs non-light-responsive units, herein "NonCRF". **D)** Electrophysiological characterization of CRF vs non-CRF neurons. Graphs showing firing rate, coefficient of variation, % of spikes in bursts, burst duration, intraburst frequency, and burst rate. Error bars are standard error of the mean. We found CRF units had a higher firing rate (U=1589, p=.005), a smaller coefficient of variation (U=1488, p=.0011), and a higher burst rate (U=1770, p=.0469) when compared to non-CRF units. **p<.01, *p<.05, Mann-Whitney test.

STATISTICS

Prism File-

Firing Rate:

1) Normal/parametric?

No, failed D'agostino & Pearson , shapiro willik tests

2) Stats Including Outliers

Mann Whitney test	
P value	0.0050
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column B,C	4439 , 4606
Mann-Whitney U	1589

Coefficient of Variation:

1) Normal/parametric?

A: No, failed both D'agostino and Shapiro-Willik

2) Stats Including Outliers

Mann Whitney test	
P value	0.0011
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column B,C	5787 , 3258
Mann-Whitney U	1488

Burst Rate

1) Normal/parametric?

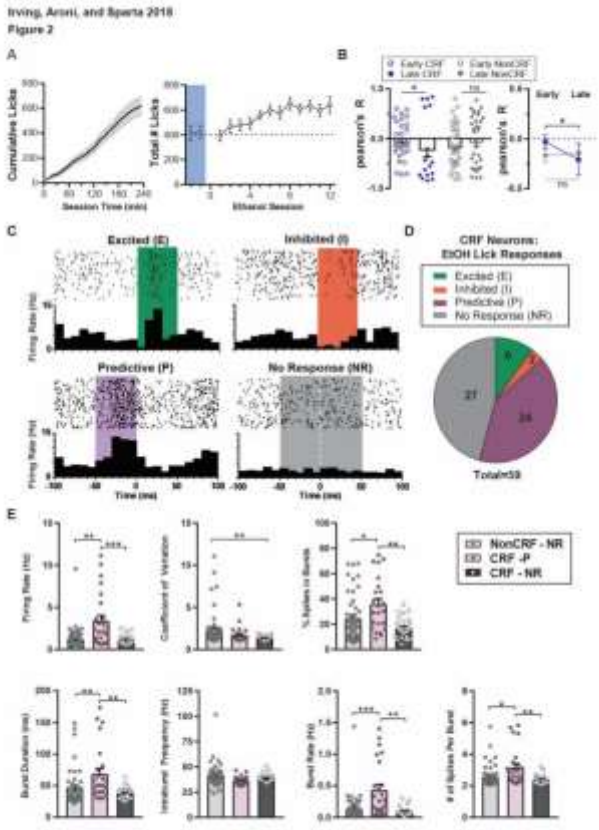
A: No, failed both

2) Stats Including Outliers

Mann Whitney test	
P value	0.0469
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column B,C	4620 , 4425
Mann-Whitney U	1770

II. FIGURE 2

JPG



LEGEND

Figure 2. CRF Neurons encode licking-behavior. **A, Left)** Average cumulative licks during a drinking session for all recorded sessions. **A, right)** Graph of representative number of licks over repeated ethanol sessions in a different cohort of mice. **B)** Graphs show that overall, CRF units had a significant shift in their correlation values for firing rate vs cumulative licks (D=0.4804, p=.0134) whereas non-CRF units did not (D=0.2267, p=.2951). *p<.05, Kolmogorov-Smirnov test. **C)** Perievent Raster plots for representative units for each lick-response type. Units were classified into 4 lick-response types, based upon changes in firing rates during 3 time periods: baseline (-100ms to -50 ms before licks) vs. pre-lick (-50 ms to 0ms), post-lick (0ms to +50ms). Wilcoxon signed-rank tests were performed on pairs of these time periods to determine if there were significant changes in firing rates from baseline to pre-lick and from pre-lick to post-lick. **D)** The pie chart indicates that out of our 59 CRF units, 6 were lick-excited (CRF-E), 2 were lick-inhibited (CRF-I), 24 were lick-predictive (CRF-P), and 27 showed no response (CRF-NR). Due to the low number of CRF-E and CRF-I units, we focused on the two major response classes, CRF-NR and CRF-P. **E)** Graph show electrophysiological parameters such as firing rate, coefficient of variation, % of spikes in bursts, burst duration, intraburst frequency, burst rate and # of spikes per burst, of CRF-NR, CRF-P, and non-CRF NR (included as a control). CRF-P units had a higher firing rate (H=16.43, p=.0003), percentage of spikes in bursts (H=12.55, p=.0019), burst duration (H=14.09, p=.0009), burst rate (H=16.8, p=.0002), and # of spikes per burst (H=11.45, p=.0033) than CRF-NR and non-CRF-NR units. Furthermore, CRF-NR cells showed a lower coefficient of variation when compared to non-CRF-NR (H=9.492, p=.0087). ***p<.001, **p<.01, *p<.05, Dunn's multiple comparisons test.

STATISTICS

Prism File – Licking Behavior (no stats)

Correlation of CRF Firing Rates [New 2B: was old Fig 4]

Prism File – Separated 09/03/18

~~Prism File #1:~~ ~~Prism File: For the CRF vs Non CRF Correlations Early vs Late~~
~~New Prism File~~

Column A	Early CRF
vs.	vs.
Column B	Late CRF
Kolmogorov-Smirnov test	
P value	0.0134
Exact or approximate P value?	Approximate
P value summary	*
Significantly different (P < 0.05)?	Yes
Kolmogorov-Smirnov D	0.4804

Table Analyzed	2B. column version of CRF vs Non Early Vs Late
Column C	Early Non-CRF
vs.	vs.
Column D	Late Non-CRF
Kolmogorov-Smirnov test	
P value	0.2951
Exact or approximate P value?	Approximate
P value summary	ns
Significantly different (P < 0.05)?	No
Kolmogorov-Smirnov D	0.2267

	Early CRF	Late CRF	Early Non-CRF	Late Non-CRF
Number of values	30	17	41	34
Mean	-0.0302	-0.2076	-0.1578	-0.05262
Std. Deviation	0.3791	0.6599	0.4124	0.5078
Std. Error of Mean	0.06921	0.1601	0.06441	0.08709
D'Agostino & Pearson normality test				
K2	1.039	4.601	2.086	23.31
P value	0.5948	0.1002	0.3524	<0.0001
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	No
P value summary	ns	ns	ns	****
Shapiro-Wilk normality test				
W	0.9638	0.7861	0.9649	0.9018
P value	0.3849	0.0013	0.2320	0.0051
Passed normality test (alpha=0.05)?	Yes	No	Yes	No
P value summary	ns	**	ns	**

Firing Rate

1) Normal/parametric?

A:All 3 fail shapiro-wilk test; Both CRF-I and CRF-NR fail D'agostino & Pearson

2) Stats Including Outliers

Kruskal-Wallis test	
P value	0.0003
Exact or approximate P value?	Approximate
P value summary	***
Do the medians vary signif. (P < 0.05)?	Yes
Number of groups	3
Kruskal-Wallis statistic	16.43

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value	
CRF-P vs. CRF-NR	24.08	Yes	**	0.0076	B-C
CRF-P vs. non-CRF-NR	28.3	Yes	***	0.0002	B-E
CRF-NR vs. non-CRF-NR	4.225	No	ns	>0.9999	C-E

% Spikes in Bursts

1) Normal/parametric?

A: No, fail both tests.

2) Stats Including Outliers

Table Analyzed	%SpikesInBurst
Kruskal-Wallis test	
P value	0.0019
Exact or approximate P value?	Approximate
P value summary	**
Do the medians vary signif. (P < 0.05)?	Yes
Number of groups	3
Kruskal-Wallis statistic	12.55

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value	
CRF-P vs. CRF-NR	27.81	Yes	**	0.0015	B-C
CRF-P vs. non-CRF-NR	18.34	Yes	*	0.0304	B-E
CRF-NR vs. non-CRF-NR	-9.469	No	ns	0.5037	C-E

Burst Duration

1) Normal/parametric?

A: No.

2) Stats Including Outliers

Kruskal-Wallis test	
P value	0.0009
Exact or approximate P value?	Approximate
P value summary	***
Do the medians vary signif. (P < 0.05)?	Yes

Number of groups	3
Kruskal-Wallis statistic	14.09

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value	
CRF-P vs. CRF-NR	26.59	Yes	**	0.0026	B-C
CRF-P vs. non-CRF-NR	23.99	Yes	**	0.0023	B-E
CRF-NR vs. non-CRF-NR	-2.6	No	ns	>0.9999	C-E

Burst Rate

1) Normal/parametric?

A: No, failed both tests

2) Stats Including Outliers

Kruskal-Wallis test	
P value	0.0002
Exact or approximate P value?	Approximate
P value summary	***
Do the medians vary signif. (P < 0.05)?	Yes
Number of groups	3
Kruskal-Wallis statistic	16.8

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value	
CRF-P vs. CRF-NR	27.06	Yes	**	0.0021	B-C
CRF-P vs. non-CRF-NR	27.55	Yes	***	0.0003	B-E
CRF-NR vs. non-CRF-NR	0.4941	No	ns	>0.9999	C-E

of Spikes Per Burst

1) Normal/parametric?

A: No

2) Stats Including Outliers

Kruskal-Wallis test	
P value	0.0033
Exact or approximate P value?	Approximate
P value summary	**
Do the medians vary signif. (P < 0.05)?	Yes
Number of groups	3
Kruskal-Wallis statistic	11.45

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value	
CRF-P vs. CRF-NR	26.55	Yes	**	0.0026	B-C
CRF-P vs. non-CRF-NR	17.59	Yes	*	0.0410	B-E
CRF-NR vs. non-CRF-NR	-8.956	No	ns	0.5764	C-E

CV

1) Normal/parametric?

A: I/NR & NR-NR all no by both D'Adostino & Pearson test as well as Shapiro-Wilik norality test

2) Stats Including Outliers

Table Analyzed	Coefficient of Variation

Kruskal-Wallis test	
P value	0.0087
Exact or approximate P value?	Approximate
P value summary	**
Do the medians vary signif. (P < 0.05)?	Yes
Number of groups	3
Kruskal-Wallis statistic	9.492

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value
CRF-P vs. CRF-NR	14.12	No	ns	0.2394
CRF-P vs. non-CRF-NR	-7.167	No	ns	0.9548
CRF-NR vs. non-CRF-NR	-21.28	Yes	**	0.0062

Intraburst Freq

1) Normal/parametric?

A:No.

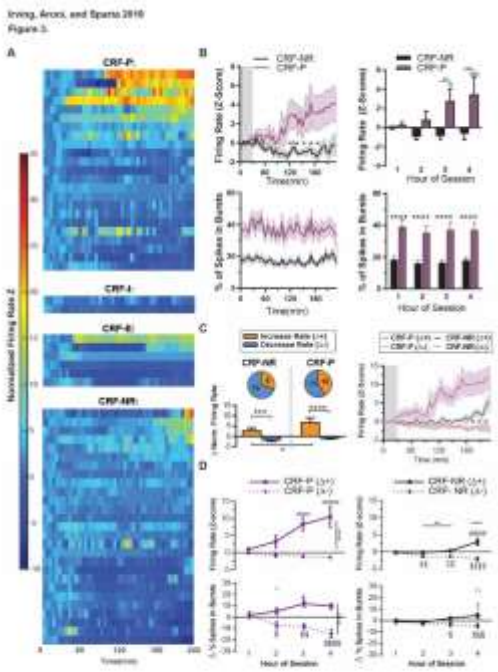
2) Stats Including Outliers

Kruskal-Wallis test	
P value	0.0529
Exact or approximate P value?	Approximate
P value summary	ns
Do the medians vary signif. (P < 0.05)?	No
Number of groups	3
Kruskal-Wallis statistic	5.88

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value	
CRF-P vs. CRF-NR	-13	No	ns	0.3095	B-C
CRF-P vs. non-CRF-NR	-17.21	Yes	*	0.0476	B-E
CRF-NR vs. non-CRF-NR	-4.206	No	ns	>0.9999	C-E

III. FIGURE 3

JPG



LEGEND

Figure 3. CRF-P Neurons increase firing activity during ethanol sessions, with heterogeneous sub-types. **A)** Normalized Firing Rate Z-Scores were calculated using the first 30 mins as the baseline period for calculating the mean and std used to calculate Z-scores for the full-session. Z-scores are shown in color, with each horizontal line is one unit's activity for the 4-hour drinking session. Units are grouped by lick-response type and then ordered from top-bottom by rate change (hour 4 – hour 1). **B) Left**: Average normalized firing rates and percentage of spikes in bursts calculated in 5 min bins. **Right**: Hourly averages of the same activity used for statistical analysis. **Right, top:** CRF-P units had a higher firing rate vs CRF-NR units (main effect of CRF type: $F_{(1, 49)} = 7.957$, p=.0069; hour: $F_{(3, 147)} = 2.545$, p=.0583; CRF-type by hour interaction ($F_{(3, 147)} = 3.924$, p=.0099). **p<.01, 2way ANOVA. Post-hoc tests show that CRF-P units increased throughout the session with significantly higher rates by hours 3 and 4 ($^{\alpha\alpha}$p<.01, $^{\alpha}$p<.05, Tukey's tests), whereas CRF-NR did not change across hours. **Right, bottom:** CRF-P units also had higher % of spikes in bursts ($F_{(1, 49)} = 21.6$, p<.0001), but did not change over the session ($F_{(3, 147)} = 1.775$, p=.1545). ****p<.0001, 2way ANOVA. **C) Left:** Pie charts and bar graphs show how units were sorted by change in firing rate (hour 4 – hour 1). CRF-NR and CRF-P were separated into two statistically different subtypes that increased firing rate (Δ+) and decreased rate (Δ-) respectively (ethanol response: $F_{(1, 46)} = 7.019$, p=.0110; CRF-type: $F_{(1, 46)} = 55.06$, p<.0001). ****p<.0001, ***p<.001, *p<.01, Sidak's multiple comparison test. **Right:** The average normalized firing of each CRF sub-types: CRF-NR(Δ+) (n=8), CRF-NR(Δ-) (n=19), CRF-P(Δ+) (n=9), CRF-P(Δ-) (n=14). **D) Left, top**: CRF-P(Δ-) and CRF-P(Δ+) firing rates were significantly distinct ($F_{(1, 22)} = 32.86$, p<.0001) for hours 3 and 4 (Sidak's test: ****p<.0001). CRF-P(Δ+) increased throughout the sessions with hours 1 and 2 distinct from 3 and 4 ($^{\#\#\#\#}$p<.0001, $^{\#\#\#}$p<.001, Tukey's tests), but CRF-P(Δ-) did not change. **Left, bottom**: CRF-P subtypes had a significantly different change in % of spikes in bursts for hours 2-4 ($F_{(1, 22)} = 22.73$, Sidak's test: ****p<.0001,) with a significant hour by sub-type interaction ($F_{(3, 66)} = 11.98$, p<.0001). CRF-P(Δ+) changed from hour 1 vs hour 3($^{\epsilon}$p<.05, Tukey's test), whereas CRF-P(Δ-) changed from hour 1 vs 2-4 ($^{\$\$\$\$}$p<.0001, $^{\$\$}$p<.01, $^{\$}$p<.05, Tukey's tests). **Right, top:** CRF-NR(Δ+) and CRF-NR(Δ-) were significantly different ($F_{(1, 26)} = 34.41$, p<.0001) for hours 2-4 (Sidak's test: ****p<.0001, **p<.01). CRF-NR(Δ+) increased firing rate by hour 4 vs hours 1-3 (Tukey's tests: ####p<.0001). CRF-NR(Δ-) had lower firing rates in hours 2-4 vs hour 1 (Tukey's test: ‡‡‡‡p<.0001, ‡‡p<.01). **Right, bottom:** While CRF-NR subtypes did not have a significant main effect of sub-type ($F_{(1, 25)} = 3.071$, p=.0920) or hour ($F_{(3, 75)} = 0.9743$, p=.4095), there was a significant

sub-type by hour interaction ($F_{(3, 75)}$ = 7.535, p=.0002). Post-hoc tests show that CRF-NR(Δ-) units decreased % of spikes in bursts from hour 1 to hours 3 and 4 (Tukey's tests: §§§p<.001, §p<.05) and CRF-NR(Δ+) units increased from hours 1 and 2 to hour 4 (Tukey's tests: †p<.05).

STATISTICS

~~Prism File~~

New Prism File

Firing Rate Z-scores [New Licks]:

Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	3.037	0.0099	**	Yes	
Time	1.97	0.0583	ns	No	
Unit Type	8.006	0.0069	**	Yes	
Subjects (matching)	49.3	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	119.2	3	39.74	F (3, 147) = 3.924	P=0.0099
Time	77.34	3	25.78	F (3, 147) = 2.545	P=0.0583
Unit Type	314.3	1	314.3	F (1, 49) = 7.957	P=0.0069
Subjects (matching)	1936	49	39.5	F (49, 147) = 3.9	P<0.0001
Residual	1489	147	10.13		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR - CRF-P					
1	-0.3055	-3.254 to 2.642	No	ns	0.9982
2	-1.816	-4.764 to 1.132	No	ns	0.4089
3	-3.682	-6.63 to -0.7342	Yes	**	0.0078
4	-4.144	-7.092 to -1.196	Yes	**	0.0020

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR					
1 vs. 2	0.9335	-1.317 to 3.184	No	ns	0.7036
1 vs. 3	0.8386	-1.412 to 3.089	No	ns	0.7677
1 vs. 4	0.5892	-1.662 to 2.84	No	ns	0.9045
2 vs. 3	-0.09493	-2.346 to 2.156	No	ns	0.9995
2 vs. 4	-0.3443	-2.595 to 1.907	No	ns	0.9786
3 vs. 4	-0.2494	-2.5 to 2.001	No	ns	0.9916
CRF-P					
1 vs. 2	-0.5765	-2.964 to 1.811	No	ns	0.9231
1 vs. 3	-2.538	-4.925 to -0.1507	Yes	*	0.0324
1 vs. 4	-3.249	-5.637 to -0.862	Yes	**	0.0030
2 vs. 3	-1.962	-4.349 to 0.4258	No	ns	0.1469
2 vs. 4	-2.673	-5.06 to -0.2855	Yes	*	0.0215
3 vs. 4	-0.7113	-3.099 to 1.676	No	ns	0.8660

% of Spikes in Bursts:

Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.03895	0.8922	ns	No	
Hour	0.3359	0.1545	ns	No	
Unit Type	27.64	<0.0001	****	Yes	
Subjects (matching)	62.72	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	27.99	3	9.329	F (3, 147) = 0.2058	P=0.8922
Hour	241.4	3	80.46	F (3, 147) = 1.775	P=0.1545
Unit Type	19865	1	19865	F (1, 49) = 21.6	P<0.0001
Subjects (matching)	45069	49	919.8	F (49, 147) = 20.29	P<0.0001
Residual	6663	147	45.33		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR - CRF-P					
Hour 1	-20.54	-31.99 to -9.077	Yes	****	<0.0001
Hour 2	-19.13	-30.59 to -7.671	Yes	***	0.0002
Hour 3	-20.48	-31.94 to -9.024	Yes	****	<0.0001
Hour 4	-18.93	-30.39 to -7.476	Yes	***	0.0002

RateSplits – Change in Rate Bar Graphs [CRF-P vs CRF-NR]

Table Analyzed	CRF-P vs CRF-NR Change in Firing Rate TRANSPOSE				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.443	0.1042	ns	No	
Ethanol Response	6.241	0.0110	*	Yes	
CRF Type	48.95	<0.0001	****	Yes	
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	24.92	1	24.92	F (1, 46) = 2.748	P=0.1042
Ethanol Response	63.66	1	63.66	F (1, 46) = 7.019	P=0.0110
CRF Type	499.3	1	499.3	F (1, 46) = 55.06	P<0.0001
Residual	417.2	46	9.069		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
EE - EI					
CRF-NR	5.207	2.273 to 8.141	Yes	***	0.0003
CRF-P	8.203	5.229 to 11.18	Yes	****	<0.0001

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR - CRF-P					
EE	-3.892	-7.275 to -0.5093	Yes	*	0.0213
EI	-0.8962	-3.348 to 1.556	No	ns	0.6430

RateSplits – Normalized Firing Rate – CRF-P

Table Analyzed	NewSplit_FIG_NormRate SPLITS - 60min bin NEW 04182018				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	13.26	<0.0001	****	Yes	
Hour	8.438	0.0005	***	Yes	
Unit Split Group	32.29	<0.0001	****	Yes	
Subjects (matching)	21.62	0.0038	**	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value

Interaction	421.4	3	140.5	F (3, 66) = 10.65	P<0.0001
Hour	268.2	3	89.41	F (3, 66) = 6.777	P=0.0005
Unit Split Group	1026	1	1026	F (1, 22) = 32.86	P<0.0001
Subjects (matching)	687.3	22	31.24	F (22, 66) = 2.368	P=0.0038
Residual	870.8	66	13.19		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-P: EI - CRF-P: EE					
1	-1.174	-5.604 to 3.256	No	ns	0.9386
2	-4.036	-8.466 to 0.3943	No	ns	0.0883
3	-9.381	-13.81 to -4.951	Yes	****	<0.0001
4	-11.94	-16.37 to -7.508	Yes	****	<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-P: EI					
1 vs. 2	0.616	-3.003 to 4.235	No	ns	0.9697
1 vs. 3	0.8815	-2.737 to 4.5	No	ns	0.9179
1 vs. 4	1.236	-2.383 to 4.854	No	ns	0.8048
2 vs. 3	0.2655	-3.353 to 3.884	No	ns	0.9974
2 vs. 4	0.6198	-2.999 to 4.238	No	ns	0.9691
3 vs. 4	0.3543	-3.264 to 3.973	No	ns	0.9939
CRF-P: EE					
1 vs. 2	-2.246	-6.528 to 2.036	No	ns	0.5146
1 vs. 3	-7.326	-11.61 to -3.044	Yes	***	0.0002
1 vs. 4	-9.529	-13.81 to -5.247	Yes	****	<0.0001
2 vs. 3	-5.08	-9.361 to -0.7979	Yes	*	0.0137
2 vs. 4	-7.283	-11.56 to -3.001	Yes	***	0.0002
3 vs. 4	-2.203	-6.485 to 2.079	No	ns	0.5311

RateSplits – Normalized Firing Rate – CRF-NR

Table Analyzed	FIG_NormRate SPLITS - 60min bin NEW 04182018				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	14.08	<0.0001	****	Yes	
Hour	3.275	0.0483	*	Yes	
Unit Split Group	29.44	<0.0001	****	Yes	
Subjects (matching)	22.24	0.0050	**	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	61.78	3	20.59	F (3, 78) = 11.82	P<0.0001
Hour	14.37	3	4.791	F (3, 78) = 2.75	P=0.0483
Unit Split Group	129.2	1	129.2	F (1, 26) = 34.41	P<0.0001
Subjects (matching)	97.62	26	3.755	F (26, 78) = 2.155	P=0.0050
Residual	135.9	78	1.742		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR: EI - CRF-NR: EE					
1	-0.2426	-1.678 to 1.193	No	ns	0.9880
2	-2.119	-3.554 to -0.6833	Yes	**	0.0012
3	-1.827	-3.263 to -0.3915	Yes	**	0.0067
4	-4.404	-5.84 to -2.969	Yes	****	<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR: EI					
1 vs. 2	1.34	0.2884 to 2.391	Yes	**	0.0068
1 vs. 3	1.392	0.3408 to 2.444	Yes	**	0.0046
1 vs. 4	2.132	1.081 to 3.184	Yes	****	<0.0001
2 vs. 3	0.05234	-0.9992 to 1.104	No	ns	0.9992
2 vs. 4	0.7921	-0.2594 to 1.844	No	ns	0.2050
3 vs. 4	0.7398	-0.3118 to 1.791	No	ns	0.2592

CRF-NR: EE					
1 vs. 2	-0.03175	-1.652 to 1.589	No	ns	>0.9999
1 vs. 3	-0.4764	-2.097 to 1.144	No	ns	0.8666
1 vs. 4	-3.075	-4.696 to -1.455	Yes	****	<0.0001
2 vs. 3	-0.4447	-2.065 to 1.176	No	ns	0.8884
2 vs. 4	-3.043	-4.664 to -1.423	Yes	****	<0.0001
3 vs. 4	-2.599	-4.219 to -0.9782	Yes	***	0.0004

RateSplits – CHANGE IN % SPIKES - CRF-P: (Δ-) vs (Δ+)

Table Analyzed	*Change*HourAvgNewSplit_percBurst_hour avg BARS				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	12.87	<0.0001	****	Yes	
Hour	2.139	0.1239	ns	No	
Unit Type	30.45	<0.0001	****	Yes	
Subjects (matching)	29.47	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1980	3	660	F (3, 66) = 11.98	P<0.0001
Hour	329	3	109.7	F (3, 66) = 1.991	P=0.1239
Unit Type	4683	1	4683	F (1, 22) = 22.73	P<0.0001
Subjects (matching)	4532	22	206	F (22, 66) = 3.74	P<0.0001
Residual	3636	66	55.09		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-P: EI - CRF-P: EE					
Hour 1	-0.04565	-10.19 to 10.1	No	ns	>0.9999
Hour 2	-12.34	-22.49 to -2.201	Yes	*	0.0105
Hour 3	-19.95	-30.09 to -9.805	Yes	****	<0.0001
Hour 4	-24.33	-34.47 to -14.19	Yes	****	<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-P: EI					
Hour 1 vs. Hour 2	8.845	1.451 to 16.24	Yes	*	0.0127
Hour 1 vs. Hour 3	9.843	2.449 to 17.24	Yes	**	0.0044
Hour 1 vs. Hour 4	16.48	9.085 to 23.87	Yes	****	<0.0001
Hour 2 vs. Hour 3	0.9985	-6.395 to 8.392	No	ns	0.9844
Hour 2 vs. Hour 4	7.635	0.2407 to 15.03	Yes	*	0.0404
Hour 3 vs. Hour 4	6.636	-0.7578 to 14.03	No	ns	0.0939
CRF-P: EE					
Hour 1 vs. Hour 2	-3.455	-12.2 to 5.294	No	ns	0.7262
Hour 1 vs. Hour 3	-10.06	-18.81 to -1.311	Yes	*	0.0179
Hour 1 vs. Hour 4	-7.806	-16.55 to 0.9428	No	ns	0.0969
Hour 2 vs. Hour 3	-6.605	-15.35 to 2.144	No	ns	0.2022
Hour 2 vs. Hour 4	-4.351	-13.1 to 4.397	No	ns	0.5594
Hour 3 vs. Hour 4	2.254	-6.495 to 11	No	ns	0.9047

EVEN WITH A ONE WAY RM ANOVA (FRIEDMAN TEST) ONLY HR1 VS HR4 for CRF-P:EE IS SIG:

Table Analyzed	*Change*HourAvgNewSplit_percBurst_hour avg BARS-TRANS
Friedman test	
P value	0.0053
Exact or approximate P value?	Approximate
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	12.72

Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value	
CRF-P: EE-Hour 1 vs. CRF-P: EE-Hour 2	-10	No	ns	0.4996	M-N
CRF-P: EE-Hour 1 vs. CRF-P: EE-Hour 3	-20	Yes	**	0.0032	M-O
CRF-P: EE-Hour 1 vs. CRF-P: EE-Hour 4	-14	No	ns	0.0919	M-P
CRF-P: EE-Hour 2 vs. CRF-P: EE-Hour 3	-10	No	ns	0.4996	N-O
CRF-P: EE-Hour 2 vs. CRF-P: EE-Hour 4	-4	No	ns	>0.9999	N-P
CRF-P: EE-Hour 3 vs. CRF-P: EE-Hour 4	6	No	ns	>0.9999	O-P

RateSplits – CHANGE IN % SPIKES - CRF-NR: (Δ-) vs (Δ+)

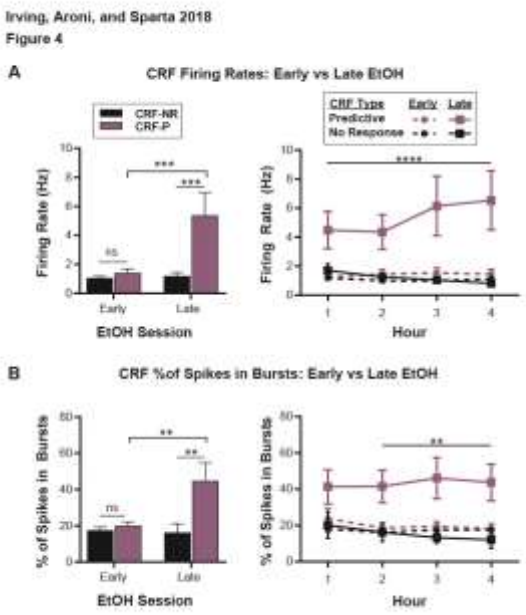
Table Analyzed	*Change*HourAvgNewSplit_percBurst_hour avg BARS					
Two-way RM ANOVA	Matching: Stacked					
Alpha	0.05					
Source of Variation	% of total variation		P value	P value summary	Significant?	
Interaction	8.784		0.0002	***	Yes	
Hour	1.136		0.4095	ns	No	
Unit Type	6.547		0.0920	ns	No	
Subjects (matching)	53.3		<0.0001	****	Yes	
ANOVA table	SS		DF	MS	F (DFn, DFd)	P value
Interaction	363.9		3	121.3	F (3, 75) = 7.535	P=0.0002
Hour	47.06		3	15.69	F (3, 75) = 0.9743	P=0.4095
Unit Type	271.3		1	271.3	F (1, 25) = 3.071	P=0.0920
Subjects (matching)	2208		25	88.32	F (25, 75) = 5.486	P<0.0001
Residual	1208		75	16.1		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR: EI - CRF-NR: EE					
Hour 1	1.566	-4.682 to 7.814	No	ns	0.9497
Hour 2	-1.116	-7.364 to 5.132	No	ns	0.9853
Hour 3	-5.376	-11.62 to 0.8724	No	ns	0.1199
Hour 4	-8.957	-15.21 to -2.709	Yes	**	0.0018

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR: EI					
Hour 1 vs. Hour 2	3.096	-0.325 to 6.516	No	ns	0.0902
Hour 1 vs. Hour 3	3.964	0.5436 to 7.385	Yes	*	0.0166
Hour 1 vs. Hour 4	5.235	1.814 to 8.655	Yes	***	0.0008
Hour 2 vs. Hour 3	0.8686	-2.552 to 4.289	No	ns	0.9091
Hour 2 vs. Hour 4	2.139	-1.282 to 5.56	No	ns	0.3612
Hour 3 vs. Hour 4	1.27	-2.15 to 4.691	No	ns	0.7636
CRF-NR: EE					
Hour 1 vs. Hour 2	0.4137	-4.858 to 5.685	No	ns	0.9969
Hour 1 vs. Hour 3	-2.977	-8.249 to 2.294	No	ns	0.4522
Hour 1 vs. Hour 4	-5.289	-10.56 to -0.01718	Yes	*	0.0490
Hour 2 vs. Hour 3	-3.391	-8.663 to 1.881	No	ns	0.3360
Hour 2 vs. Hour 4	-5.703	-10.97 to -0.4309	Yes	*	0.0288
Hour 3 vs. Hour 4	-2.312	-7.583 to 2.96	No	ns	0.6586

IV. FIGURE 4

JPG



LEGEND

Figure 4. Changes in CRF activity over repeated ethanol sessions. **A)** CRF-P increased raw firing rates after repeated ethanol sessions (main effect of ethanol session: $F_{(1, 38)} = 11.34$, p=.0017; main effect of CRF type: $F_{(1, 38)} = 13.85$, p=.0006; Tukey's tests: Early vs Late CRF-P: ***p=.0005), which was absent in CRF-NR units (Tukey's tests: Early vs Late CRF-NR: p=.9986). **B)** Similarly, CRF-P units showed a significant increase in the percentage of spikes in bursts after repeated ethanol sessions (main effect of CRF-type: $F_{(1, 38)} = 12.44$, p=.0011, ethanol session: $F_{(1, 38)} = 7.138$, p=.0111; Tukey's tests: Early vs Late CRF-P: **p=.0029) and CRF-NR did not (Tukey's tests, p=.9961).

STATISTICS

~~New Prism File~~

Prism File – Data Family Separated 09/03/18

Fig 4A, Bar: CRF-P vs CRF-NR: Early vs Late - Firing Rate (Hz)

Table Analyzed	NewLicks*FIG_CRF-NR vs CRF- Firing Rate - Early vs late 05022018				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	13.86	0.0031	**	Yes	
Ethanol session	15.77	0.0017	**	Yes	
Cell Type	19.25	0.0006	***	Yes	
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	30.48	1	30.48	F (1, 38) = 9.967	P=0.0031
Ethanol session	34.69	1	34.69	F (1, 38) = 11.34	P=0.0017
Cell Type	42.35	1	42.35	F (1, 38) = 13.85	P=0.0006
Residual	116.2	38	3.058		

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Early:CRF-NR vs. Early:CRF-P	-0.3424	-2.294 to 1.61	No	ns	0.9649
Early:CRF-NR vs. Late:CRF-NR	**-0.1279**	**-2.303 to 2.047**	**No**	**ns**	**0.9986**
Early:CRF-NR vs. Late:CRF-P	-4.304	-6.354 to -2.254	Yes	****	<0.0001
Early:CRF-P vs. Late:CRF-NR	0.2145	-2.323 to 2.752	No	ns	0.9958
Early:CRF-P vs. Late:CRF-P	**-3.962**	**-6.393 to -1.53**	**Yes**	****	**0.0005**
Late:CRF-NR vs. Late:CRF-P	-4.176	-6.79 to -1.562	Yes	***	0.0007

Fig 4A, Line: CRF-P vs CRF-NR: Early vs Late By Hour: Firing Rate (Hz)

Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.7	<0.0001	****	Yes	
Hour	0.4995	0.0639	ns	No	
Unit Type	42.14	<0.0001	****	Yes	
Subjects (matching)	47.2	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	26.59	9	2.954	F (9, 114) = 4.484	P<0.0001
Hour	4.919	3	1.64	F (3, 114) = 2.489	P=0.0639
Unit Type	415.1	3	138.4	F (3, 38) = 11.31	P<0.0001
Subjects (matching)	464.9	38	12.23	F (38, 114) = 18.57	P<0.0001
Residual	75.11	114	0.6588		

UPDATED POST HOC [Discussed @ meeting on 08/09/18]

Within each row, compare columns (simple effects within rows)					
Number of families	4				
Number of comparisons per family	6				
Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Hour 1					
CRF-NR: Early Days vs. CRF-NR: Late Days	-0.5162	-2.783 to 1.75	No	ns	0.9345
CRF-NR: Early Days vs. CRF-P: Early	-0.06464	-2.099 to 1.97	No	ns	0.9998
CRF-NR: Late Days vs. CRF-P: Late	-2.785	-5.509 to -0.06102	Yes	*	0.0430
CRF-P: Early vs. CRF-P: Late	-3.237	-5.771 to -0.7025	Yes	**	0.0062
Hour 2					
CRF-NR: Early Days vs. CRF-NR: Late Days	-0.2523	-2.519 to 2.014	No	ns	0.9915
CRF-NR: Early Days vs. CRF-P: Early	-0.452	-2.486 to 1.582	No	ns	0.9388
CRF-NR: Late Days vs. CRF-P: Late	-3.104	-5.828 to -0.38	Yes	*	0.0185

CRF-P: Early vs. CRF-P: Late	-2.904	-5.438 to -0.3702	Yes	*	0.0176				
Hour 3									
CRF-NR: Early Days vs. CRF-NR: Late Days	-0.05317	-2.32 to 2.213	No	ns	>0.9999				
CRF-NR: Early Days vs. CRF-P: Early	-0.5399	-2.574 to 1.494	No	ns	0.9010				
CRF-NR: Late Days vs. CRF-P: Late	-5.101	-7.825 to -2.377	Yes	****	<0.0001				
CRF-P: Early vs. CRF-P: Late	-4.614	-7.148 to -2.08	Yes	****	<0.0001				
Hour 4									
CRF-NR: Early Days vs. CRF-NR: Late Days	0.31	-1.956 to 2.577	No	ns	0.9846				
CRF-NR: Early Days vs. CRF-P: Early	-0.3131	-2.347 to 1.721	No	ns	0.9783				
CRF-NR: Late Days vs. CRF-P: Late	-5.715	-8.439 to -2.991	Yes	****	<0.0001				
CRF-P: Early vs. CRF-P: Late	-5.092	-7.626 to -2.558	Yes	****	<0.0001				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF	
Hour 1									
CRF-NR: Early Days vs. CRF-NR: Late Days	1.185	1.701	-0.5162	0.8725	21	6	0.8367	152	
CRF-NR: Early Days vs. CRF-P: Early	1.185	1.249	-0.06464	0.7831	21	8	0.1167	152	
CRF-NR: Late Days vs. CRF-P: Late	1.701	4.486	-2.785	1.049	6	7	3.756	152	
CRF-P: Early vs. CRF-P: Late	1.249	4.486	-3.237	0.9755	8	7	4.692	152	
Hour 2									
CRF-NR: Early Days vs. CRF-NR: Late Days	0.9963	1.249	-0.2523	0.8725	21	6	0.4089	152	
CRF-NR: Early Days vs. CRF-P: Early	0.9963	1.448	-0.452	0.7831	21	8	0.8163	152	
CRF-NR: Late Days vs. CRF-P: Late	1.249	4.352	-3.104	1.049	6	7	4.186	152	
CRF-P: Early vs. CRF-P: Late	1.448	4.352	-2.904	0.9755	8	7	4.21	152	
Hour 3									
CRF-NR: Early Days vs. CRF-NR: Late Days	0.9882	1.041	-0.05317	0.8725	21	6	0.08619	152	
CRF-NR: Early Days vs. CRF-P: Early	0.9882	1.528	-0.5399	0.7831	21	8	0.975	152	
CRF-NR: Late Days vs. CRF-P: Late	1.041	6.142	-5.101	1.049	6	7	6.879	152	
CRF-P: Early vs. CRF-P: Late	1.528	6.142	-4.614	0.9755	8	7	6.689	152	
Hour 4									
CRF-NR: Early Days vs. CRF-NR: Late Days	1.128	0.8182	0.31	0.8725	21	6	0.5025	152	
CRF-NR: Early Days vs. CRF-P: Early	1.128	1.441	-0.3131	0.7831	21	8	0.5654	152	
CRF-NR: Late Days vs. CRF-P: Late	0.8182	6.533	-5.715	1.049	6	7	7.707	152	
CRF-P: Early vs. CRF-P: Late	1.441	6.533	-5.092	0.9755	8	7	7.381	152	

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR: Early Days					
Hour 1 vs. Hour 2	0.1884	-0.4647 to 0.8416	No	ns	0.8755
Hour 1 vs. Hour 3	0.1965	-0.4567 to 0.8496	No	ns	0.8615
Hour 1 vs. Hour 4	0.05643	-0.5967 to 0.7096	No	ns	0.9959
Hour 2 vs. Hour 3	0.008019	-0.6451 to 0.6611	No	ns	>0.9999
Hour 2 vs. Hour 4	-0.132	-0.7851 to 0.5211	No	ns	0.9524
Hour 3 vs. Hour 4	-0.14	-0.7931 to 0.5131	No	ns	0.9439
CRF-NR: Late Days					
Hour 1 vs. Hour 2	0.4524	-0.7695 to 1.674	No	ns	0.7694
Hour 1 vs. Hour 3	0.6595	-0.5624 to 1.881	No	ns	0.4975
Hour 1 vs. Hour 4	0.8826	-0.3392 to 2.105	No	ns	0.2409
Hour 2 vs. Hour 3	0.2071	-1.015 to 1.429	No	ns	0.9710
Hour 2 vs. Hour 4	0.4303	-0.7916 to 1.652	No	ns	0.7952
Hour 3 vs. Hour 4	0.2232	-0.9987 to 1.445	No	ns	0.9642
CRF-P: Early					
Hour 1 vs. Hour 2	-0.1989	-1.257 to 0.8593	No	ns	0.9611
Hour 1 vs. Hour 3	-0.2788	-1.337 to 0.7794	No	ns	0.9019
Hour 1 vs. Hour 4	-0.192	-1.25 to 0.8662	No	ns	0.9649
Hour 2 vs. Hour 3	-0.07986	-1.138 to 0.9783	No	ns	0.9973
Hour 2 vs. Hour 4	0.006927	-1.051 to 1.065	No	ns	>0.9999
Hour 3 vs. Hour 4	0.08679	-0.9714 to 1.145	No	ns	0.9965
CRF-P: Late					
Hour 1 vs. Hour 2	0.1334	-0.9978 to 1.265	No	ns	0.9899
Hour 1 vs. Hour 3	1.656	2.787 to -0.525	Yes	**	0.0012
Hour 1 vs. Hour 4	2.047	3.178 to -0.9158	Yes	****	<0.0001
Hour 2 vs. Hour 3	1.79	2.921 to -0.6584	Yes	***	0.0004
Hour 2 vs. Hour 4	2.18	3.312 to -1.049	Yes	****	<0.0001
Hour 3 vs. Hour 4	0.3908	-1.522 to 0.7405	No	ns	0.8045

Commented [JI1]: ONLY CRF-P UNITS FROM LATE SESSIONS SHOW CHANGES IN FIRING RATE ACROSS HOURS

Fig 4B, Bars: CRF-P vs CRF-NR: Early vs Late - % Spikes In Bursts

Table Analyzed	FIG_CRF-NR vs CRF-P PercBurst- Early vs late -Bar				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	13.62	0.0052	**	Yes	
EarlyVsLate	11.03	0.0111	*	Yes	
Unit Type	19.23	0.0011	**	Yes	
ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	1412	1	1412	F (1, 38) = 8.808	P=0.0052
EarlyVsLate	1144	1	1144	F (1, 38) = 7.138	P=0.0111
Unit Type	1994	1	1994	F (1, 38) = 12.44	P=0.0011
Residual	6090	38	160.3		

Compare cell means regardless of rows and columns					
Number of families	1				
Number of comparisons per family	6				
Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Early:CRF-NR vs. Early:CRF-P	-2.459	-16.59 to 11.67	No	ns	0.9657
Early:CRF-NR vs. Late:CRF-NR	1.302	-14.44 to 17.05	No	ns	0.9961
Early:CRF-NR vs. Late:CRF-P	-27.25	-42.09 to -12.4	Yes	****	<0.0001
Early:CRF-P vs. Late:CRF-NR	3.761	-14.61 to 22.13	No	ns	0.9460
Early:CRF-P vs. Late:CRF-P	-24.79	-42.39 to -7.185	Yes	**	0.0029
Late:CRF-NR vs. Late:CRF-P	-28.55	-47.47 to -9.626	Yes	**	0.0013

Fig 4B, Line: CRF-P vs CRF-NR: Early vs Late - % Spikes In Bursts

Table Analyzed	FIG_CRF-NR vs CRF-P PercBurst- Early vs late - LINES				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.8743	0.4817	ns	No	
Hour	0.3907	0.2849	ns	No	
Unit Type	34.89	0.0002	***	Yes	
Subjects (matching)	52.31	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	389.5	9	43.28	F (9, 114) = 0.9542	P=0.4817
Hour	174.1	3	58.02	F (3, 114) = 1.279	P=0.2849
Unit Type	15541	3	5180	F (3, 38) = 8.447	P=0.0002
Subjects (matching)	23304	38	613.3	F (38, 114) = 13.52	P<0.0001
Residual	5170	114	45.35		

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
CRF-NR: Early Days					
1 vs. 2	1.862	-3.557 to 7.281	No	ns	0.8069
1 vs. 3	0.4754	-4.943 to 5.894	No	ns	0.9958
1 vs. 4	0.4337	-4.985 to 5.852	No	ns	0.9968
2 vs. 3	-1.387	-6.805 to 4.032	No	ns	0.9092
2 vs. 4	-1.428	-6.847 to 3.99	No	ns	0.9018
3 vs. 4	-0.04172	-5.46 to 5.377	No	ns	>0.9999
CRF-NR: Late Days					
1 vs. 2	3.837	-6.3 to 13.97	No	ns	0.7571
1 vs. 3	6.92	-3.217 to 17.06	No	ns	0.2883
1 vs. 4	8.007	-2.131 to 18.14	No	ns	0.1729
2 vs. 3	3.083	-7.055 to 13.22	No	ns	0.8576
2 vs. 4	4.169	-5.968 to 14.31	No	ns	0.7071
3 vs. 4	1.087	-9.051 to 11.22	No	ns	0.9923
CRF-P: Early					
1 vs. 2	5.096	-3.683 to 13.88	No	ns	0.4329
1 vs. 3	4.335	-4.444 to 13.11	No	ns	0.5728
1 vs. 4	5.58	-3.199 to 14.36	No	ns	0.3511
2 vs. 3	-0.7611	-9.54 to 8.018	No	ns	0.9959
2 vs. 4	0.4846	-8.295 to 9.264	No	ns	0.9989
3 vs. 4	1.246	-7.534 to 10.02	No	ns	0.9826
CRF-P: Late					
1 vs. 2	-0.1146	-9.5 to 9.271	No	ns	>0.9999
1 vs. 3	-4.709	-14.09 to 4.676	No	ns	0.5596
1 vs. 4	-2.346	-11.73 to 7.039	No	ns	0.9147
2 vs. 3	-4.594	-13.98 to 4.791	No	ns	0.5798
2 vs. 4	-2.232	-11.62 to 7.154	No	ns	0.9255
3 vs. 4	2.363	-7.023 to 11.75	No	ns	0.9131

UPDATED POST HOC [Discussed @ meeting on 08/09/18]

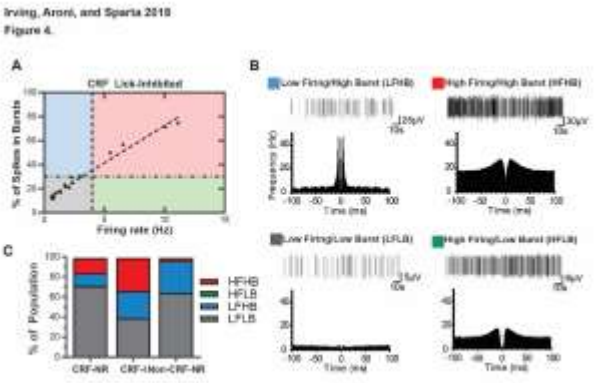
Within each row, compare columns (simple effects within rows)					
Number of families	4				
Number of comparisons per family	6				
Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
1					
CRF-NR: Early Days vs. CRF-NR: Late Days	-2.115	-18.57 to 14.34	No	ns	0.9871
CRF-NR: Early Days vs. CRF-P: Early	-5.727	-20.5 to 9.044	No	ns	0.7455
CRF-NR: Late Days vs. CRF-P: Late	-21.24	-41.02 to -1.462	Yes	*	0.0300
CRF-P: Early vs. CRF-P: Late	-17.63	-36.03 to 0.7704	No	ns	0.0657
2					
CRF-NR: Early Days vs. CRF-NR: Late Days	-0.1397	-16.6 to 16.32	No	ns	>0.9999
CRF-NR: Early Days vs. CRF-P: Early	-2.493	-17.27 to 12.28	No	ns	0.9717

CRF-NR: Late Days vs. CRF-P: Late	-25.19	-44.97 to -5.414	Yes	**	0.0064
CRF-P: Early vs. CRF-P: Late	-22.84	-41.24 to -4.44	Yes	**	0.0083
3					
CRF-NR: Early Days vs. CRF-NR: Late Days	4.33	-12.13 to 20.79	No	ns	0.9033
CRF-NR: Early Days vs. CRF-P: Early	-1.868	-16.64 to 12.9	No	ns	0.9877
CRF-NR: Late Days vs. CRF-P: Late	-32.87	-52.65 to -13.09	Yes	***	0.0002
CRF-P: Early vs. CRF-P: Late	-26.67	-45.08 to -8.273	Yes	**	0.0013
4					
CRF-NR: Early Days vs. CRF-NR: Late Days	5.458	-11 to 21.92	No	ns	0.8247
CRF-NR: Early Days vs. CRF-P: Early	-0.5805	-15.35 to 14.19	No	ns	0.9996
CRF-NR: Late Days vs. CRF-P: Late	-31.6	-51.38 to -11.82	Yes	***	0.0003
CRF-P: Early vs. CRF-P: Late	-25.56	-43.96 to -7.156	Yes	**	0.0023

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
1								
CRF-NR: Early Days vs. CRF-NR: Late Days	17.99	20.11	-2.115	6.336	21	6	0.4721	152
CRF-NR: Early Days vs. CRF-P: Early	17.99	23.72	-5.727	5.686	21	8	1.424	152
CRF-NR: Late Days vs. CRF-P: Late	20.11	41.35	-21.24	7.615	6	7	3.945	152
CRF-P: Early vs. CRF-P: Late	23.72	41.35	-17.63	7.084	8	7	3.52	152
2								
CRF-NR: Early Days vs. CRF-NR: Late Days	16.13	16.27	-0.1397	6.336	21	6	0.03119	152
CRF-NR: Early Days vs. CRF-P: Early	16.13	18.62	-2.493	5.686	21	8	0.6201	152
CRF-NR: Late Days vs. CRF-P: Late	16.27	41.46	-25.19	7.615	6	7	4.679	152
CRF-P: Early vs. CRF-P: Late	18.62	41.46	-22.84	7.084	8	7	4.56	152
3								
CRF-NR: Early Days vs. CRF-NR: Late Days	17.51	13.19	4.33	6.336	21	6	0.9664	152
CRF-NR: Early Days vs. CRF-P: Early	17.51	19.38	-1.868	5.686	21	8	0.4645	152
CRF-NR: Late Days vs. CRF-P: Late	13.19	46.06	-32.87	7.615	6	7	6.105	152
CRF-P: Early vs. CRF-P: Late	19.38	46.06	-26.67	7.084	8	7	5.325	152
4								
CRF-NR: Early Days vs. CRF-NR: Late Days	17.56	12.1	5.458	6.336	21	6	1.218	152
CRF-NR: Early Days vs. CRF-P: Early	17.56	18.14	-0.5805	5.686	21	8	0.1444	152
CRF-NR: Late Days vs. CRF-P: Late	12.1	43.69	-31.6	7.615	6	7	5.868	152
CRF-P: Early vs. CRF-P: Late	18.14	43.69	-25.56	7.084	8	7	5.102	152

V. FIGURE S1

JPG



LEGEND

We further analyzed the firing/burst properties of the units neurons by classifying them into four different groups (using a cutoff of firing rate to 4 Hz, and burst firing to 30%. The four classes were low firing/high burst (LFHB), high firing/high burst (HFHB), low firing/low burst (LFLB), and high firing/low burst (HBLB). **A)** Example spike trains and autocorrelograms from representative units of each class. **B)** Units plotted as % of spikes in bursts vs firing rate. The horizontal dashed line marks the cutoff between low and high bursting (cutoff=30%), and the vertical dashed line marks the cutoff between low and high firing (cutoff=4 Hz). **C)** Population distributions for the burst-firing class for the lick-response types of interest. CRF-P had approximately 26% of LFHB, 23% of HFHB, and 50% of LFLB, whereas CRF-NR showed a low % of LFHB (7%), no HFHB, and a higher % of LFLB (~92%). NonCRF non-lick responsive (NR-NR) units had ~75% LFLB, and 25% LFHB, and a very low % of HFHB units (2%), indicating the HFHB class may be a defining characteristic for CeA-CRF-P units