3.3.2 Analytical Approach for Assessing Differential Patterns of FosTRAP Activation

Because we injected 4-OHT during the initial stress exposure (Fig. 2A), the persistently tagged td-Tomato (FosTRAP+) cells represent the neural patterns that were activated during that first experience. Using the cell detection and registration tool NeuroInfo (MBF Bioscience), we were able to quantify these activated cells by counting the total number of FosTRAP+ cells per region in a high-throughput manner. As a result, our dependent variable in all these analyses is the total number of FosTRAP+ cells per region.

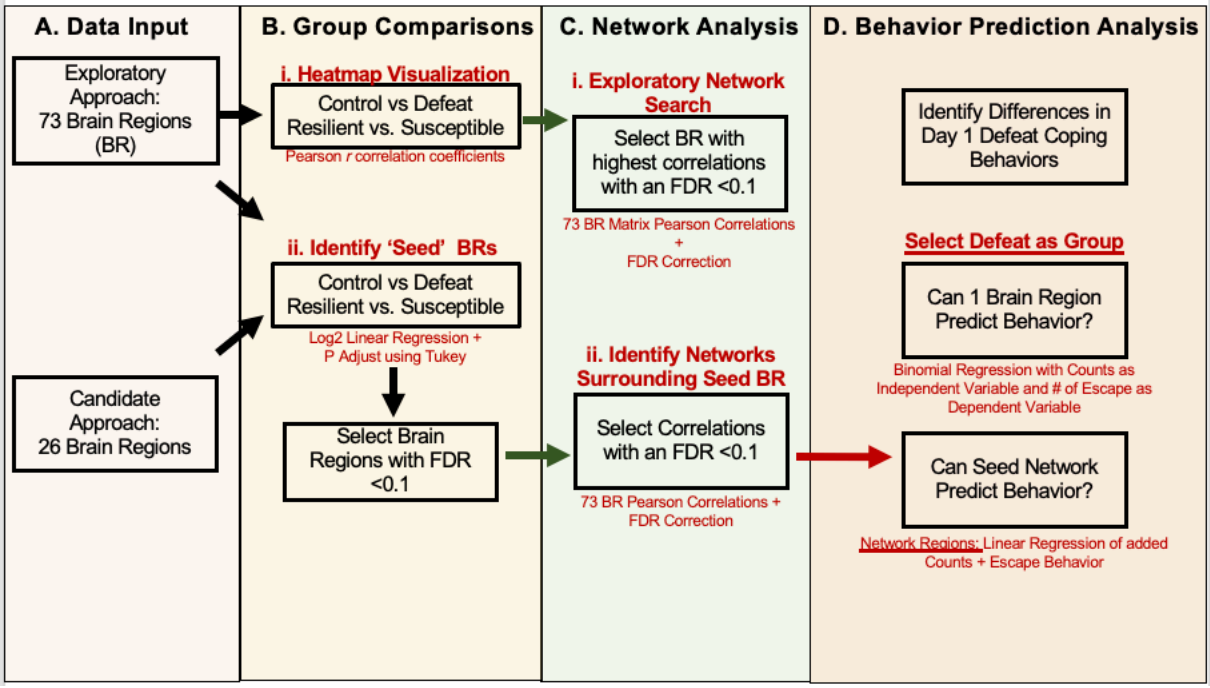

Figure 3.3 Data Analysis Pipeline
Data Analysis was split into 4 sections A. Data Input, B. Group Comparisons, C. Network Analysis, and D. Behavior Prediction Analysis

3.3.3 Data Input: Brain Regions Selected Based on an Exploratory and a Candidate Approach

To assess differences between the initial Control vs. Defeat, as well as future Resilient vs. Susceptible populations, we took on two different approaches in inputting our data (**Fig. 3.3A**). Our first level of analysis was to use an unbiased exploratory approach: We selected 73 brain regions (See **Appendix B. Table B1**) based on any region that had FosTRAP activity (count of >1) during the stress or social experience on Day 1. Our second level of analysis was to take a candidate approach: We selected 26 brain regions (See **Appendix B. Table B2**) based on circuitry that had been previously identified in the CSDS model, as well as areas known in the literature to be involved in stress and social responses. These 26 brain regions were a subset of the overall 73.

Both methods have their advantages and disadvantages. In the exploratory analysis, we may identify brain regions that were not *a priori* known to be involved in the stress or social response (**Fig. 3.3B.i.**). In this case, we may see correlations of activation patterns (networks) that are either anatomically connected or not (**Fig 3.3C.i.**). In addition, multiple comparisons of this large dataset may result in very few or any brain regions meeting an FDR cut-off of 0.1(**Fig. 3.3B, C**). In our candidate approach, while we may not identify any 'new' brain regions that are engaged by CSDS, there is a higher chance of detecting correlations between regions (network activation patterns) that are known to be anatomically connected and involved in a relevant social stress response (**Fig. 3.3B.ii., C.ii.**). Moreover, focusing on 26 instead of 73 brain areas would allow us to gain more statistical power when correcting for the false discovery rate. In the results below, we will walk through examples of using either the exploratory or candidate approach for our initial group comparisons. Overall, it appears that either route results in the same significantly upregulated brain regions in Control vs. Defeat, as well as future Resilient vs.

Susceptible populations. To that end, we focused the rest of our analyses using the exploratory, 73 brain regions.

3.3.4 B.i. Group Comparisons: Patterns of Neuronal Activation Across Brain Regions within Experimental Groups

We first asked whether there were visual differences in networks of activation between our Control and Defeat condition, as well as our future Resilient and Susceptible populations. To do so, we took the Pearson *r* correlations between all 73 brain regions to visualize the overall differences in networks of activation between groups. As this was a purely visual analysis, there was no *p*-value cut-off or corrections for false discovery rate. The correlation matrix and subsequent heatmap represent the visual correlations that are relevant in both the candidate and exploratory seed approach **(Fig. 3.4)**

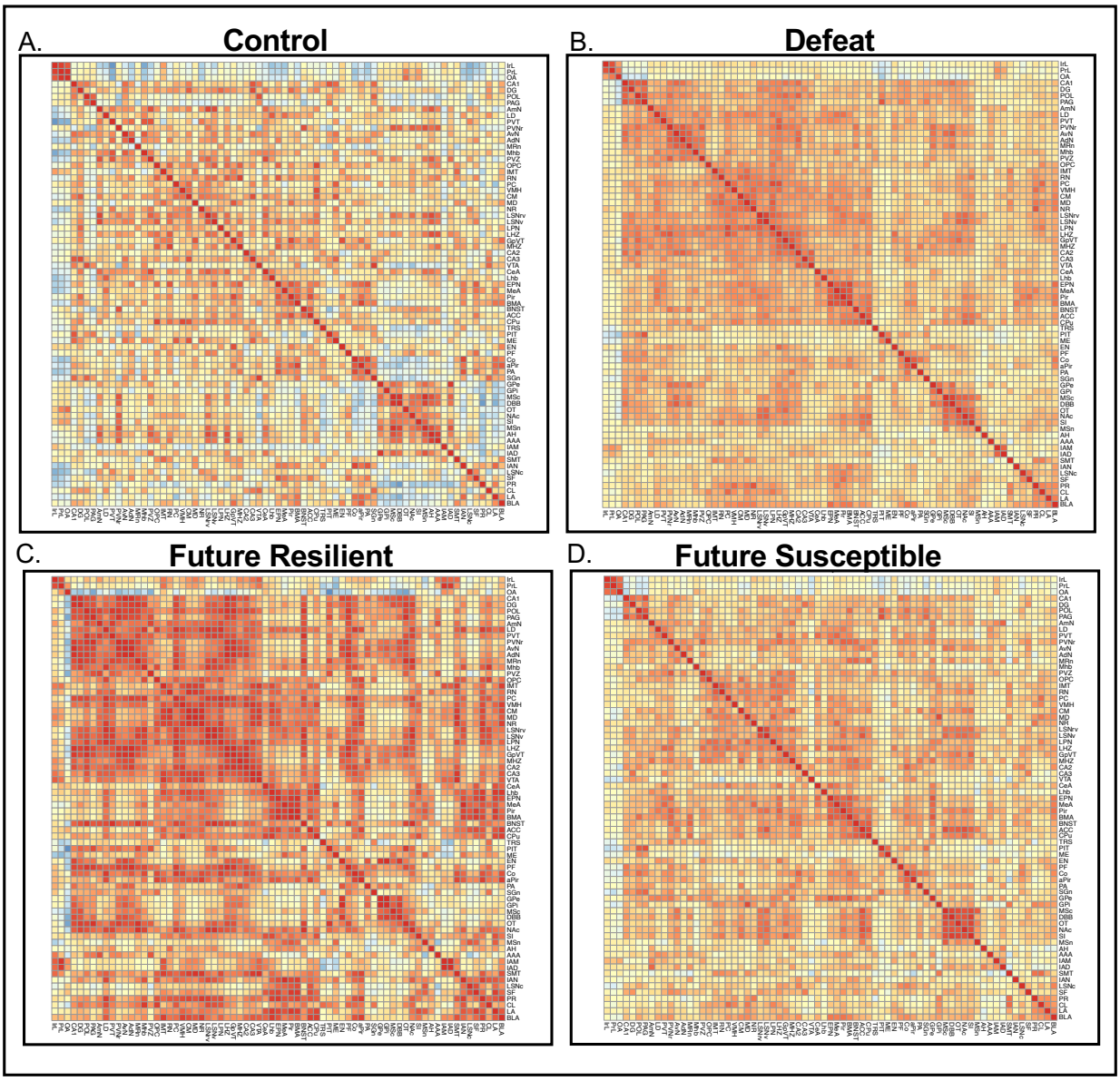

Figure 3.4 Heatmap Visualization of Correlations within A. Control, B. All Defeated, C. Future Resilient, and D. Future Susceptible mice.

Colors in heatmap indicate a range of Pearson *r* correlations, more red=1, more blue=-1. Order of brain regions was based on a clustering of all groups combined. All Pearson *r* correlations are based on total number of FosTRAP+ cells within each group for each brain region. Each row represents a brain region and its Pearson correlation with other brain regions.

Social Defeat Alters Activity Across a Large Number of Brain Regions

While Control mice do not experience Defeat, they still experience the sensory social contact of being placed across from a novel conspecific. In contrast, Defeated mice experience both the physical and psychosocial stress from the Defeat and sensory contact of the CD1 aggressor. We wanted to first assess whether there were visual differences in networks of activation between the initial Control condition (social) and Defeat (social stress). To do so, we took the Pearson *r* correlations between all brain regions to visualize overall differences in the network between groups. On day 1 of Defeat or Control rotation, defeated mice exhibited a more coordinated network of brain region activations, as indicated by the increased positive Pearson *r* values (more red) (**Fig. 3.4A, B).**

Resilient Mice Exhibit a More Coordinated Network of Activation than Susceptible Mice:

Within the Defeated group, future Resilient mice appear to have a highly increased positive correlation between brain regions in comparison to Susceptible (increased red, positive Pearson *r* values) (**Fig. 3.4C, D)**. This was not due to the differences in number of animals in each group, as a random selection of n=5 from each group condition results in a similar visual pattern (**Fig. 3.5)**. See table in appendix for list of brain regions and corresponding *p* and *r* values. Overall, it appears that the initial Defeat encounter results in an increase correlation network of activation, and this is primarily driven by the high increase of correlations within the Resilient group.

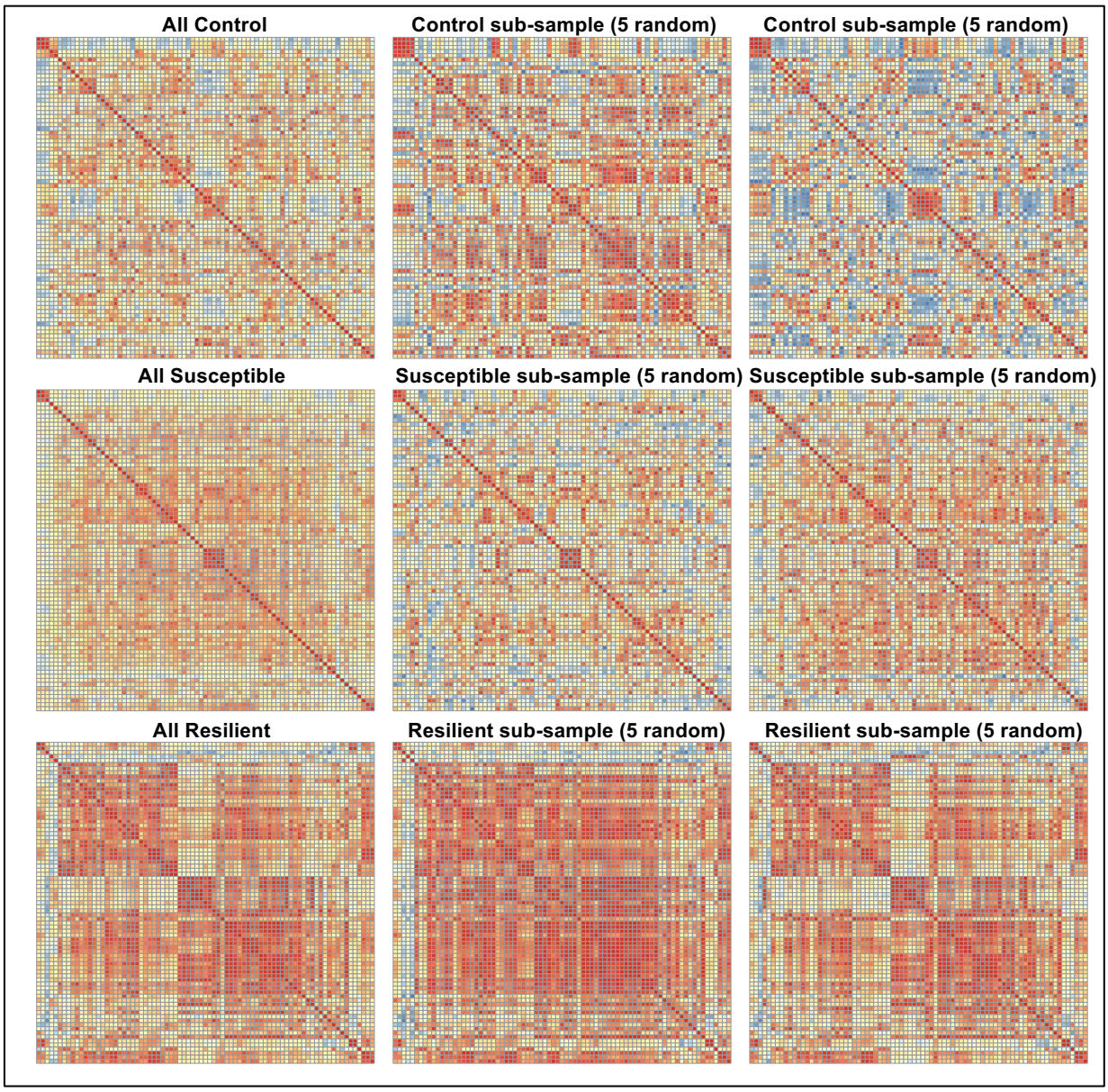

Figure 3.5 Heatmap of Sub-Sampling 5 Random Animals Per Group
Visual patterns are similar to what is observed when all animals are included, indicating that the low sample size within the Resilient animals represents a real pattern of increased activation compared to Susceptible and Control groups. Colors in heatmap indicate a range of Pearson *r* correlations, more red=1, more blue=-1.

3.3.5 C-i. An Exploratory Network Within Group Network Analysis Reveals Differential Networks of Activation Between Future Resilient and Susceptible Mice

Our first network-level approach was to analyze the correlation profiles within each group to determine which brain region emerges as the most significantly correlated (**Fig. 3.3**

C.i.) To do so, we created a matrix of Pearson *r* correlations of all 73 brain regions within each group and ran a Benjamini-Hoshberg correction on the dataset to correct for multiple comparisons. As such, we selected this exploratory seed based on highest number of brain regions that correlated with the seed and met an FDR cut-off of 0.1.

Within the Control group, the Lateral Septal Nucleus, rostroventral part (LSNrv) was the most significantly correlated with other areas. Within the defeated group, the Lateral Septal Nucleus, ventral part (LSNv) was the most significantly correlated region. This indicates that the lateral septum (LS) plays a significant role in Day 1 stress and social behaviors and possibly differentially mediated by sub-regions of the LS (*data will be expanded further upon in section 3.3.6)*.

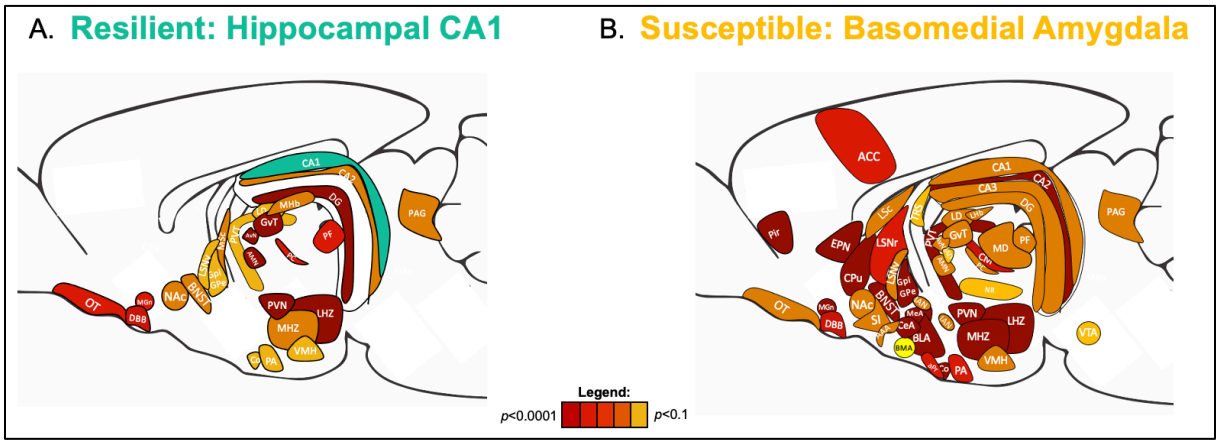

Figure 3.6 CA1 and BMA Future Resilient and Susceptible Exploratory Seed Network Maps
A. Brain regions correlating with the future Resilient CA1 seed (seed is in blue-green). B. Brain regions correlating with the future Susceptible BMA seed (seed is in bright yellow). These networks reflect a range of significantly correlated brain regions with seeds, from p <0.001 (red) to p<0.1 (yellow).

Importantly, this analysis revealed that Future Resilient and Susceptible populations exhibited distinct patterns of correlations, and the most significantly within-group correlations that emerged are known to be involved in mood-related disorders. In Future Resilient mice, the hippocampal CA1 (CA1) region correlated significantly with 22 brain regions (**Fig 3.6A)** In Future Susceptible mice, the basomedial amygdala (BMA) correlated significantly with 52 brain

regions **Fig 3.6B**). This suggests that there are separate hubs that exhibit different patterns of correlations that may prove predictive of the Resilient and Susceptible outcomes.

3.3.6 B.ii.-Quantitative Differences in FosTRAP+ Activity Between Experimental Groups

While it is visually apparent that these groups differ in terms of their correlational activation patterns, our next interest was to determine whether there were brain regions that were selectively activated by Defeat in comparison to Control. As we wanted to statistically compare multiple brain regions across groups, we ran a log2 transformation of the FosTRAP+ cell counts and ran a linear regression then corrected for multiple comparisons using the Tukey-HSD test between Control and Defeated mice in both the exploratory and candidate approach (See Appendix B **Table 1 & 2** for list of regions). The exploratory approach was done to potentially identify novel brain regions that are activated by either the Control or Defeat condition, and the candidate approach was conducted to identify whether regions traditionally associated with social and stress circuitry are differentially activated. Here, the candidate approach also acts as a proof-of-concept check that stress circuitry is being activated by an intense, stressful experience.

As mentioned in our data analysis pipeline section, the total counts across the brain between Control and Defeat, as well as Future Resilient and Susceptible groups did not differ in either the exploratory or candidate approach (**Fig. 3.7Ai,ii;** Control vs. Defeat $p>0.05$, Control vs. Resilient $p>0.05$, Control vs. Susceptible $p>0.05$, Resilient vs. Susceptible $p>0.05$).

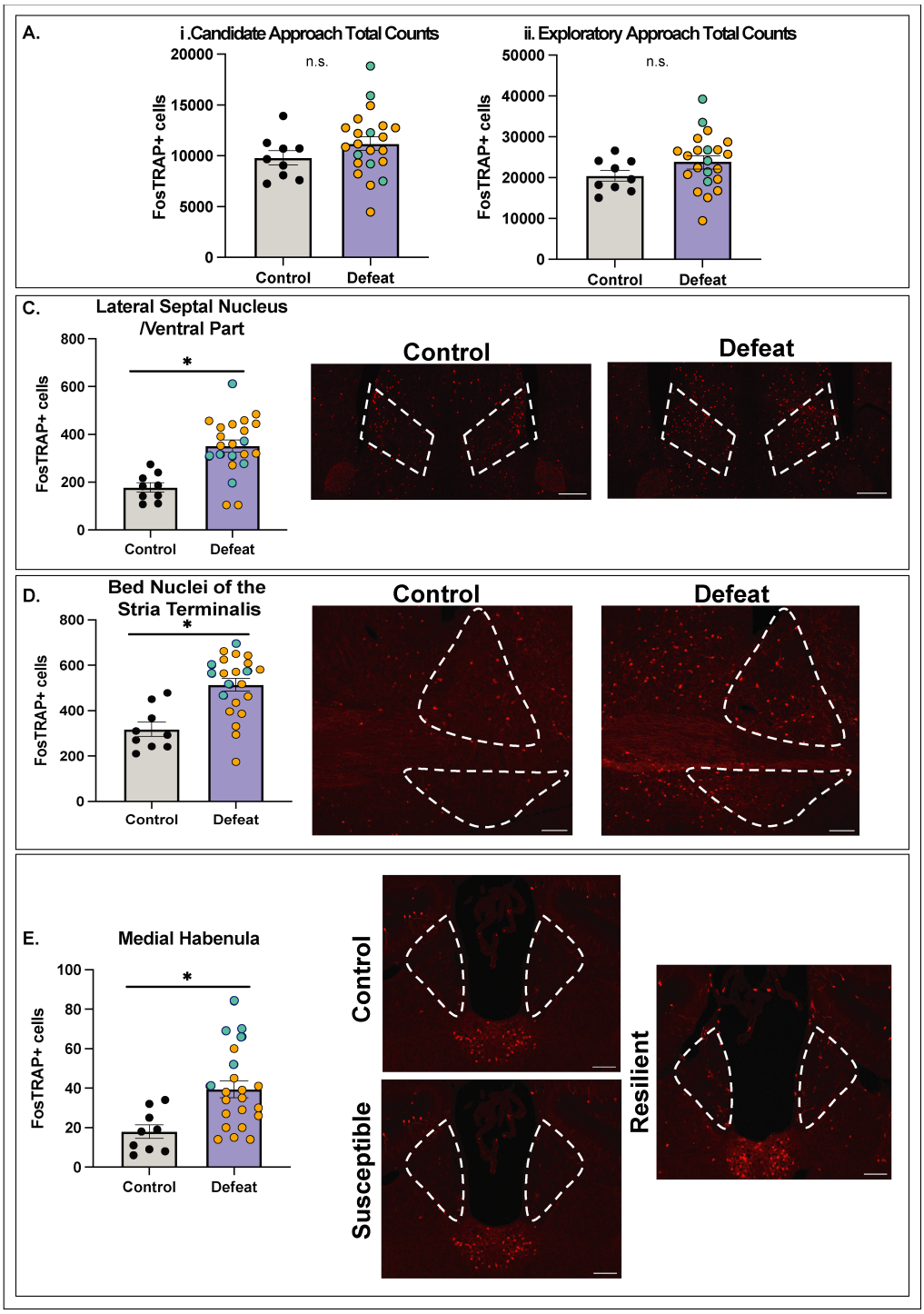

Figure 3.7 Brain Regions Selectively Active on Day 1
A. Total counts from the exploratory (73 brain regions) or candidate (26 brain regions) approach between Defeat and Control, and Future Resilient and Susceptible groups. There were no significant differences between Control vs. Defeat or Control vs. Resilient or Control vs. Susceptible or Susceptible vs. Resilient ($p>0.05$). B. Defeated animals had an increase of FosTRAP+ compared to controls (imaged at 4X). C. Defeated animals had an increase of FosTRAP+ cells in the BNST compared to controls (imaged at 10X). D. Resilient animals had an increase of FosTRAP+ cells compared to control and susceptible animals (Imaged at 10X). All scale bars are 100µm and all images were taken in stacks and Z-projected. Brain Regions of interest are outlined with white dashed line.

The LSNv and Bed Nucleus of the Stria Terminalis are the Most Differentially Active Brain Regions in Control vs. Defeat

Exploratory Approach:

In comparing Defeated vs. Control mice, out of the 73 brain regions examined, there were 6 regions that were nominally significantly different (P< 0.05) in terms of number of FosTRAP positive neurons (See **Table 3.1**). However, only 2 out of these brain regions met an FDR cut-off of below 0.05: An increase in the LSNv (p=0.004, FDR=0.025) and Bed Nucleus of the Stria Terminalis (BNST) (p=0.0007, FDR=0.025) was seen in Defeat compared to Controls. These differences are depicted in **Figure 3.7C and D.**

Table 3.1 Top 10 Brain Regions from Exploratory Approach

Region	Estimate	SE	df	t.ratio	P.value	FDR
LSNv	0.9423	0.2369	29	3.9781	0.0004	0.0249
BNST	0.6861	0.1804	29	3.8028	0.0007	0.0249
MHZ	0.5021	0.1693	29	2.9652	0.0060	0.0918
AH	0.6421	0.2179	29	2.9461	0.0063	0.0918
MHb	1.0836	0.3820	29	2.8368	0.0082	0.1001
PVN	0.5607	0.2093	29	2.6788	0.0120	0.1256
EPN	0.4073	0.2008	29	2.0282	0.0518	0.4486
CA1	0.6090	0.3050	29	1.9968	0.0553	0.4486
MeA	0.3296	0.1719	29	1.9172	0.0651	0.4753

Red meets FDR cut-off, Brown is nominally significant, and Black is non-significant.

Candidate Approach:

In our candidate approach comprising 24 brain regions, the top 2 significantly different regions between Control and Defeated mice that met an FDR cut-off of 0.05 were also the LSNv (p=0.000, FDR=0.01) and BNST (p=0.000, FDR=0.017) **(**see **Table 3.2)**. The medial Habenula (MHb) met nominal significance but did not pass FDR cut-off.

Table 3.2 Top 3 Brain Regions from Candidate Approach in Control vs. Defeat

Region	Estimate	SE	df	t.ratio	P.value	FDR
LSNv	0.9165	0.2326	29	3.9389	0.0005	0.0122
BNST	0.6798	0.1787	29	3.8042	0.0007	0.0170
MHb	0.9738	0.3479	29	2.7985	0.0090	0.2167

Red meets FDR cut-off, Brown is nominally significant, and Black is non-significant.

The Medial Habenula is the Most Differentially Activate Region in Future Resilient vs. Susceptible Mice

Exploratory Approach:

Using the same analysis parameters as above, we asked whether there were brain regions that were selective for future stress reactivity on Day 1 of Defeat. In our comparison of 73 brain regions, there were none that met an FDR cut-off of 0.1, although 4 regions were nominally significant, with the Medial Habenula (MHb) showing the largest elevation in FosTRAP+ cells in Resilient compared to Susceptible mice (**Table 3.3)**. Indeed, the overall significant increase in FosTRAP produced by Defeat depicted in the bar graph in **Fig. 3.7A.ii** is due primarily to the consistent elevation in FosTRAP+ expression the mice that will emerge as Resilient.

Table 3.3 Top 10 Brain Regions from Exploratory Approach in Future Resilient and Susceptible Mice

Region	Estimate	SE	df	t.ratio	P.value	FDR
MHb	1.0912	0.3685	20	2.9615	0.0077	0.3491
MRn	1.0063	0.3815	20	2.6380	0.0158	0.3491
GPe	0.8319	0.3258	20	2.5538	0.0189	0.3491
PVZ	0.8853	0.3474	20	2.5487	0.0191	0.3491
AdN	1.1447	0.5793	20	1.9760	0.0621	0.7761
PA	-0.7664	0.3906	20	-1.9624	0.0638	0.7761
AvN	0.7263	0.4236	20	1.7141	0.1019	0.8153
AAA	0.9886	0.6003	20	1.6469	0.1152	0.8153
GPi	0.9474	0.6055	20	1.5647	0.1333	0.8513
DBB	0.4475	0.2873	20	1.5577	0.1350	0.8513

Brown is nominally significant. Black is non-significant

Candidate Approach:

In the candidate approach, the Medial Habenula was also significantly different between groups and met the FDR cut-off threshold of 0.1, with Resilient showing greater numbers of FosTRAP+ positive neurons than Susceptible mice (**Table 3.4, Fig. 3.7E;** p=0.003, FDR=0.0731). Our results indicate that future Resilient mice selectively activate the MHb in comparison to future Susceptible mice during the initial Defeat encounter.

Table 3.4 Top 3 Brain Regions from Candidate Approach in Future Resilient Mice

Region	Estimate	SE	df	t.ratio	P.value	FDR
MHb	1.0887	0.2966	28	3.6703	0.00281	0.0731
PVZ	0.8806	0.3225	28	2.7302	0.02828	0.7071
CeA	0.4861	0.3033	28	1.6028	0.2611	1

Red meets FDR cut-off,Brown is nominally significant. Black is non-significant

3.3.7 C.ii.- Network Analyses Using the BNST, LSNv, and MHb as Seeds:

While we identified brain regions that were selectively activated by Defeat or future Resilient populations, we next wanted to ask whether there were networks of brain regions that significantly activated along with these highly upregulated regions. Activation in one area of the brain is highly unlikely to be an isolated event, and therefore uncovering what other regions potentially activate in unison may reveal some circuit-specific differences. To that end, in the Control and Defeat conditions, we selected the LSNv and BNST as 'seeds' and ran a Pearson correlation analysis between one of these seed regions and the other 73 brain regions. Next, we selected the brain regions that were most highly correlated with each seed region based on an FDR of <0.1.

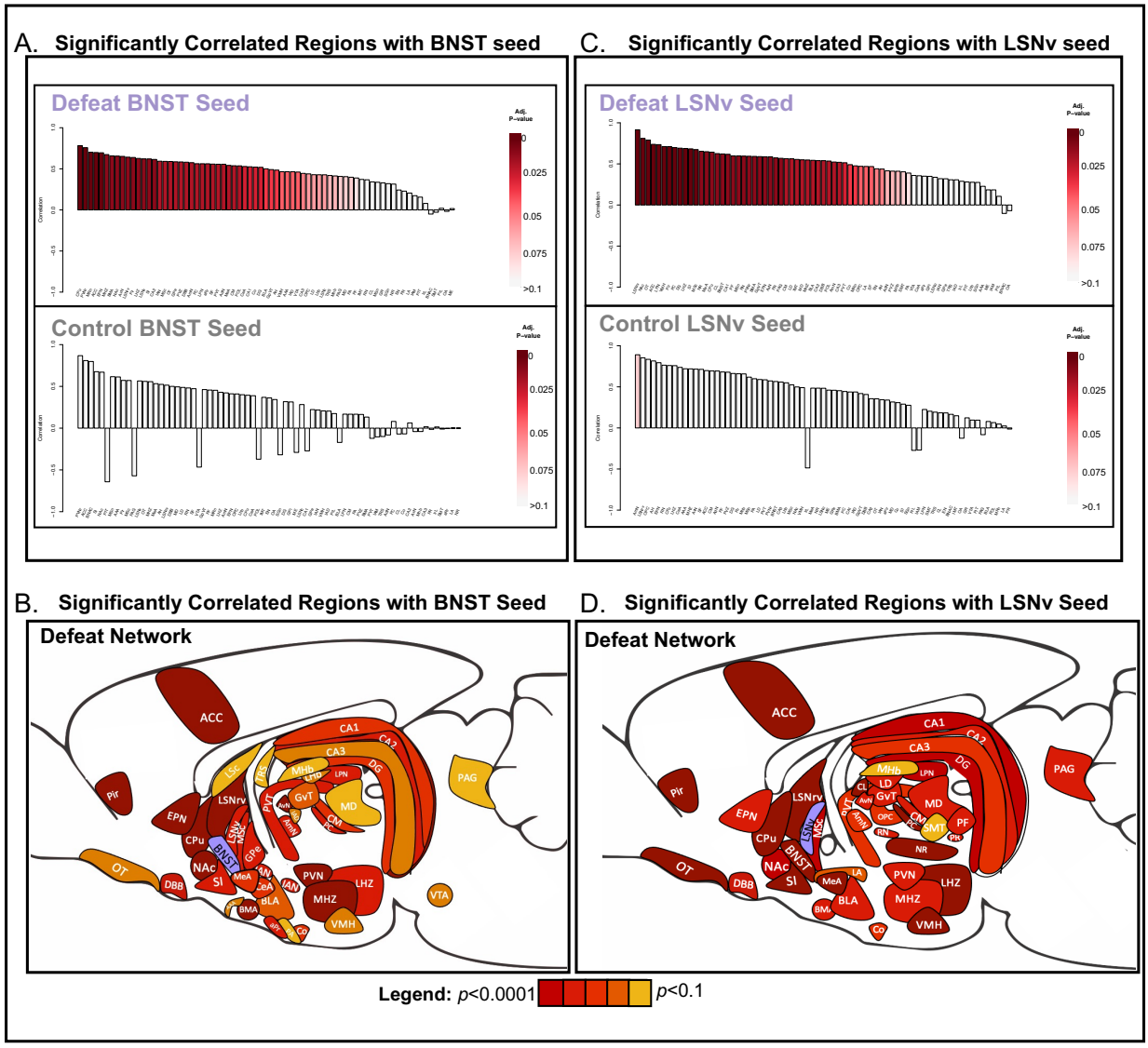

Figure 3.8 Significantly Correlated Brain Regions with LSNv and BNST Seed Networks
A. BNST Pearson R correlations of brain regions ranked based on adjusted p-values. B. Correlation network visualization of the BNST seed with all other significantly correlated regions in Defeated mice C. LSNv Pearson R correlations of brain regions ranked based on adjusted p-values D. Correlation network visualization of the LSNv seed with all other significantly correlated regions in Defeated mice. Color Code: Purple represents the defeat seed, and brain regions range from dark red ($p<0.0001$) to yellow ($p<0.1$) based on FDR significance.

The BNST Reveals a Significantly Correlated Network of Activation in Defeated mice

In a visual representation of Pearson r correlations, it appears that the BNST in Control and Defeated mice significantly correlate with different brain regions (**Fig. 3.8A**). Within Defeated mice, 52 brain regions met an FDR cut-off of 0.1 and significantly correlated with the

BNST (see **Table 3.5)**. In contrast, in Control mice, while 4 brain regions were nominally significant, there were no correlations between the BNST and other brain regions that met an FDR cut-off of 0.1 (see **Table 3.6)**. This is consistent with the fact that BNST activity was selectively enhanced by Defeat and suggests that the BNST was a centerpiece in an entire network of activation triggered by the Defeat stress. Most notably, in the Day 1 Defeat condition, the hippocampus, thalamic, hypothalamic, striatal, septal, olfactory, and cortical areas were significantly correlated with BNST activity (see **Table 3.5** below for full list and statistics). Overall, it appears that, compared to controls, defeated mice engage an additional network of activation that appears to be highly integrated with the BNST.

Table 3.5 Top Brain Regions Correlated with the BNST in Defeated Mice

Region	Correlation	P.Value	FDR
CPu	0.7823	0.0000	0.0012
PVNr	0.7576	0.0000	0.0016
MRn	0.7018	0.0003	0.0047
ACC	0.6984	0.0003	0.0047
EPN	0.6954	0.0003	0.0047
MHZ	0.6736	0.0006	0.0071
BMA	0.6575	0.0009	0.0082
NAc	0.6552	0.0009	0.0082
AvN	0.6514	0.001	0.0082
LSNrv	0.6418	0.0013	0.0091
Pir	0.6384	0.0014	0.0091
LHZ	0.6268	0.0018	0.0106
LSNv	0.6214	0.002	0.0106
SI	0.6206	0.0021	0.0106
CA2	0.6146	0.0023	0.0112
IAN	0.593	0.0036	0.0156
MSc	0.5909	0.0038	0.0156
OT	0.5894	0.0039	0.0156
GPe	0.5856	0.0042	0.0156
PVZ	0.5839	0.0043	0.0156
DBB	0.5808	0.0046	0.0157
AmN	0.575	0.0051	0.0167
PC	0.5625	0.0064	0.0185
LPN	0.5621	0.0065	0.0185

aPir	0.5617	0.0065	0.0185
SF	0.5578	0.007	0.0185
PVT	0.5561	0.0072	0.0185
AdN	0.556	0.0072	0.0185
MeA	0.5418	0.0092	0.0228
CM	0.5377	0.0099	0.0234
POL	0.5364	0.0101	0.0234
CeA	0.5285	0.0115	0.0258
CA1	0.5251	0.0121	0.0264
Co	0.5205	0.013	0.0275
DG	0.5184	0.0135	0.0277
BLA	0.5005	0.0177	0.0354
GpVT	0.491	0.0203	0.0395
AH	0.4844	0.0223	0.0423
VMH	0.465	0.0292	0.0518
AAA	0.4647	0.0293	0.0518
IAD	0.4644	0.0295	0.0518
VTA	0.4609	0.0309	0.0529
CA3	0.4446	0.0382	0.0639
OPC	0.4386	0.0412	0.0674
LD	0.4284	0.0467	0.0724
Lhb	0.4278	0.047	0.0724
LSNc	0.4274	0.0473	0.0724
TRS	0.419	0.0523	0.0784
Mhb	0.4138	0.0556	0.0816
PAG	0.4076	0.0597	0.0859
MD	0.405	0.0615	0.0869
PA	0.3978	0.0667	0.0924
PF	0.3887	0.0738	0.1003

Red meets FDR cut-off, Brown is nominally significant. Black is non-significant

Table 3.6 Top Brain Regions Correlated with the BNST in Control Mice

Region	Correlation	P.Value	FDR
PVNr	0.8678	0.0024	0.1746
ACC	0.8090	0.0083	0.2383
SI	0.6760	0.0456	0.6098
NAc	0.6703	0.0487	0.6098
PIT	-0.6421	0.0622	0.6098
MSn	0.6161	0.0773	0.6098
AAA	0.6131	0.0792	0.6098
Pir	0.5717	0.1078	0.6098

Red meets FDR cut-off, Brown is nominally significant. Black is non-significant

The LSNv Reveals a Significantly Correlated Network of Activation in Defeated mice

In a visual representation of Pearson *r* correlations, it appears that the LSNv in Control and Defeated mice have different patterns and strengths of correlations **(Fig. 3.8B)**. Within Defeated mice, 53 brain regions met an FDR cut-off of 0.1 and significantly correlated with the LSNv (see **Table 3.7)**. In contrast, in Control mice, while 20 brain regions were nominally significant, there only 1 brain region between the LSNv and other brain regions met an FDR cut-off of 0.1 (see **Table 3.8)**. Here again, the Defeat group showed a selective increase in LSNv activity compared to Controls, and that region is nodal to a network of activation that is unique to that group.

In the Day 1 Defeat condition, the hippocampus, thalamic, hypothalamic, striatal, septal, olfactory, and anterior cingulate cortex were significantly correlated with LSNv activity (see **Table 3.7** below for full list and statistics). Overall, it appears that relative to Controls, defeated mice have engaged a highly coordinated network that appears with the LSNv.

Table 3.7 Top Brain Regions Correlated with the LSNv in Defeated Mice

Region	Correlation	P.Value	FDR
LSNrv	0.9153	0.0000	0.0000
NAc	0.8105	0.0000	0.0002
OT	0.7902	0.0000	0.0003
ACC	0.738	0.0000	0.0014
LPN	0.7346	0.0000	0.0014
VMH	0.7112	0.0002	0.0021
Pir	0.7108	0.0002	0.0021
PC	0.6986	0.0003	0.0027
DG	0.6914	0.0004	0.0029
LHZ	0.6877	0.0004	0.0029
SI	0.6831	0.0005	0.003

MSc	0.6741	0.0006	0.0035
NR	0.6532	0.0010	0.0054
MeA	0.6485	0.0011	0.0056
CPu	0.6428	0.0013	0.006
CL	0.6252	0.0019	0.0084
BNST	0.6214	0.0020	0.0086
CA1	0.6167	0.0022	0.0089
PF	0.5978	0.0033	0.0114
MSn	0.5976	0.0033	0.0114
RN	0.5951	0.0035	0.0114
PVNr	0.593	0.0036	0.0114
BMA	0.5911	0.0038	0.0114
GpVT	0.5888	0.0039	0.0114
EPN	0.5868	0.0041	0.0114
AvN	0.5866	0.0041	0.0114
PR	0.5733	0.0053	0.0141
PAG	0.5679	0.0058	0.015
CM	0.564	0.0063	0.0154
LD	0.5625	0.0064	0.0154
IMT	0.5545	0.0074	0.0172
MD	0.5497	0.008	0.0181
MHZ	0.5473	0.0084	0.0183
BLA	0.5436	0.0089	0.0189
CA2	0.5399	0.0095	0.0192
DBB	0.5394	0.0096	0.0192
POL	0.5349	0.0103	0.0201
AmN	0.5229	0.0125	0.0237
CA3	0.5195	0.0132	0.0244
PVT	0.5139	0.0144	0.026
Co	0.4909	0.0204	0.0358
MRn	0.4766	0.0249	0.0428
OPC	0.4705	0.0271	0.0451
LA	0.4689	0.0277	0.0451
SF	0.4677	0.0282	0.0451
EN	0.4408	0.0401	0.0627
AH	0.4371	0.0419	0.0642
AdN	0.4156	0.0544	0.0816
PVZ	0.4118	0.0569	0.0828
Mhb	0.4109	0.0575	0.0828

SMT	0.4037	0.0624	0.0881
PA	0.3887	0.0738	0.1022
VTA	0.3595	0.1004	0.1363

Red meets FDR cut-off, Brown is nominally significant, and Black is non-significant

Table 3.8 Top Brain Regions Correlated with the LSNv in Control Mice

Region	Correlation	P.Value	FDR
AmN	0.8906	0.0013	0.0912
LSNrv	0.8534	0.0034	0.1223
OPC	0.8350	0.0051	0.1223
AH	0.8153	0.0074	0.1334
EPN	0.7939	0.0106	0.1529
RN	0.7638	0.0166	0.1614
CPu	0.7599	0.0175	0.1614
LHZ	0.7581	0.0179	0.1614
CeA	0.7371	0.0235	0.1717
AAA	0.7202	0.0287	0.1717
MHZ	0.719	0.029	0.1717
AvN	0.7155	0.0302	0.1717
SF	0.7132	0.031	0.1717
ACC	0.6979	0.0366	0.1736
CM	0.6934	0.0383	0.1736
AdN	0.6928	0.0386	0.1736
PF	0.6811	0.0434	0.1780
PVZ	0.6786	0.0445	0.1780
DG	0.6627	0.0517	0.1859

Red meets FDR cut-off, Brown is nominally significant, and Black is non-significant

While 53 brain regions were significantly correlated with either the BNST or LSNv seeds, there appeared to be an ~80% overlap between both networks. This is further emphasized by the fact that both regions are significantly correlated with each other (r=0.6214, FDR=0.0106 BNST-LSNv or 0.0009 LSNv-BNST). Despite this overlap, there are differences in strength of correlations. For example, the Nucleus Accumbens (NAc) is highly correlated in both BNST and LSNv Defeat conditions, but between the LSNv the r value is 0.812, while between the BNST the r value is 0.655. It is therefore apparent that we have identified brain-wide coordinated activity that engages a highly coordinated Defeat-specific network.

Selective Network within Future Resilient Mice Appears within the Medial Habenula (MHb)

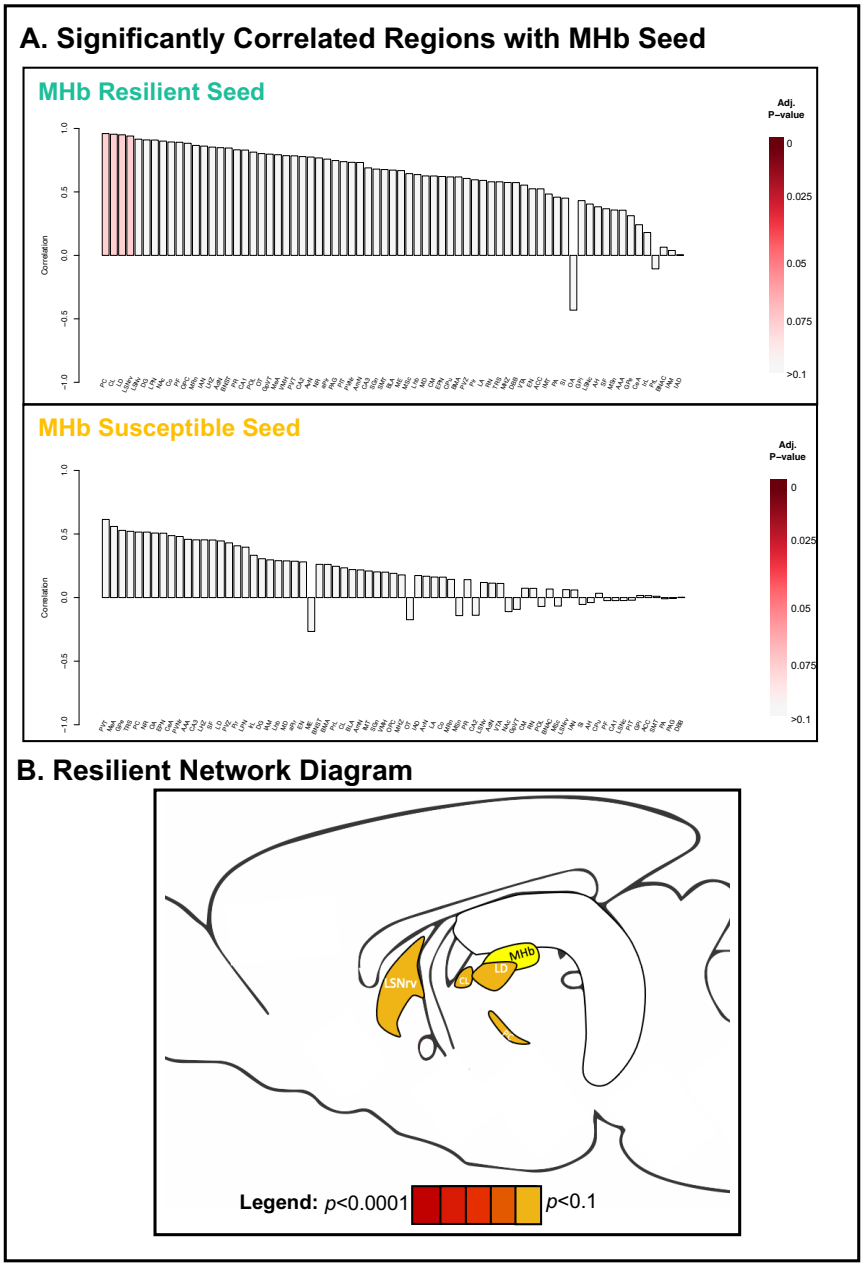

Figure 3.9 Significantly Correlated Brain Regions with MHb Seed
A. MHb Pearson R correlations of brain regions ranked based on adjusted p-values in future Resilient and Susceptible mice. B. Correlation network visualization of the significantly correlated regions with the MHb seed within future Resilient mice

In a visual representation of Pearson *r* correlations, it appears that the MHb in future Susceptible and Resilient mice have different strengths and levels of significance across brain

regions **(Fig. 3.9A)**. Within Resilient mice, 4 brain regions met an FDR cut-off of 0.1 and significantly correlated with the MHb (**Fig. 3.9B;** see **Table 3.9**). In contrast, in Control mice, while 8 brain regions were nominally significant, there were no correlations between the MHb and other brain regions that met an FDR cut-off of 0.1 (see **Table 3.10**). Overall, the MHb showed a selective increase in activity in the future Resilient group compared to Susceptible, and this increase led to a unique network of activation that is specific to future resilience to stress.

Table 3.9 Top Brain Regions Correlated with the MHb in Future Resilient

Region	Correlation	P.Value	FDR
PC	0.9597	0.0024	0.0997
CL	0.9544	0.0031	0.0997
LD	0.9497	0.0037	0.0997
LSNrv	0.9402	0.0053	0.0997
LSNv	0.9154	0.0104	0.1180
DG	0.9096	0.0119	0.1180
LPN	0.9085	0.0122	0.1180
NAc	0.9002	0.0145	0.1180
Co	0.8934	0.0165	0.1180
PF	0.8911	0.0171	0.1180
OPC	0.8832	0.0197	0.1180
MRn	0.8663	0.0256	0.1418
IAN	0.8609	0.0277	0.1424
LHZ	0.8529	0.0309	0.1432
AdN	0.8484	0.0327	0.1432
BNST	0.8458	0.0338	0.1432
PR	0.8317	0.0401	0.1556
CA1	0.8296	0.0411	0.1556
POL	0.8134	0.049	0.1763
OT	0.8018	0.055	0.1867

Red meets FDR cut-off, Brown is nominally significant, and Black is non-significant

Table 3.10. Top Brain Regions Correlated with the MHb in Future Susceptible Mice

Region	Correlation	P.Value	FDR
PVT	0.6146	0.0113	0.4023
MeA	0.5595	0.0242	0.4023
GPe	0.5284	0.0354	0.4023
TRS	0.5211	0.0385	0.4023
PC	0.5151	0.0411	0.4023
NR	0.5149	0.0413	0.4023
OA	0.5075	0.0448	0.4023
EPN	0.5063	0.0454	0.4023
CeA	0.4872	0.0556	0.4023
PVNr	0.4804	0.0596	0.4023

Brown is nominally significant, and Black is non-significant

3.3.8 D-Relationships Between Behavior and Neural Patterns of Activation on Day 1

In addition to 'TRAP'ing the circuit on Day 1 of Defeat, we quantified active and passive coping patterns during the agonistic encounter. To that end, we observed the number of fights, escapes, and number of upright, forward, and crouch-back freezing behaviors to assess the proportion of each of these coping strategies displayed by a given animal. The goal was to then 1) assess whether differences in coping strategy appeared on Day 1 between future Resilient and Susceptible mice as we had previously seen; and 2) assess whether these coping strategies mapped on to differences in brain region activation patterns during the initial Defeat encounter. Overall, we wanted to determine whether neural activation patterns and coping strategies on Day 1 could be used to predict the development of future vulnerability to social stress.

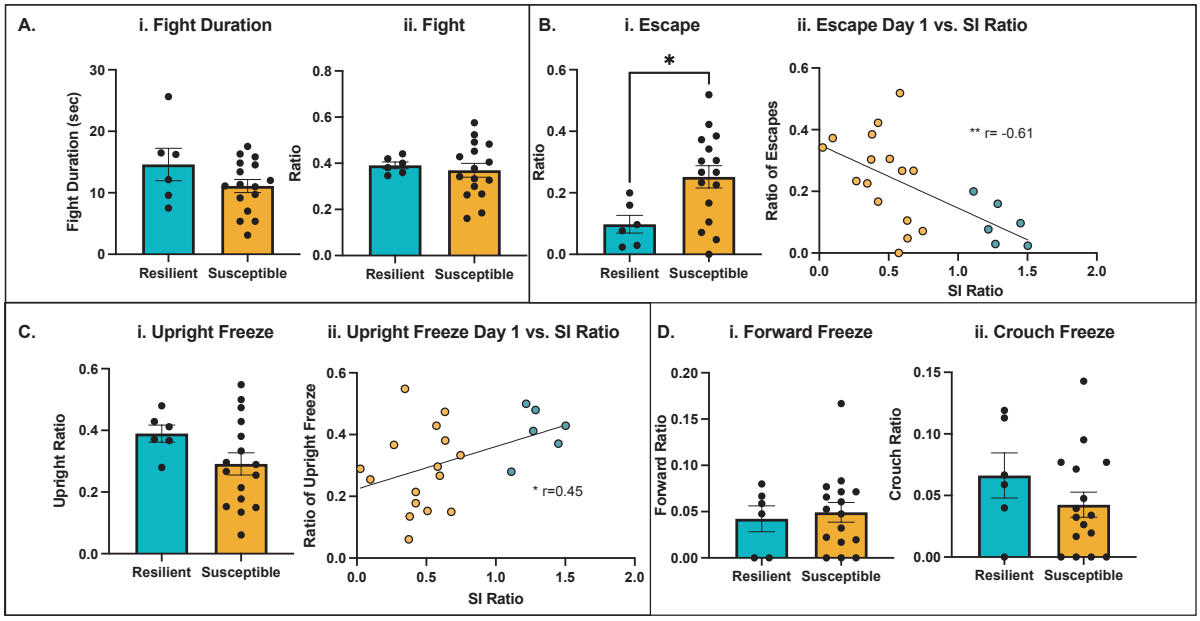

Figure 3.10 Ratio of Coping Behaviors During the Initial Defeat Encounter in Future Resilient and Susceptible Mice.

A. i. Fight Duration (sec), p>0.05, ii.# of Fights/Total Behaviors p>0.05, B. i.# of Escapes/Total Behaviors, p=0.021, ii, Escape Correlates significantly with SI Ratio. All freeze behaviors do not differ as a group between Resilient and Susceptible mice, but Cii. Upright Freeze Correlates positively correlates with SI Ratio p=0.038. C.i. # of Upright Freezes/Total Behaviors p>0.05, Di. # of Forward Freezes/Total Behaviors p>0.05, D.ii. # of Crouch Freezes p>0.05.

Colors: Blue= future Resilient, Yellow= Future Susceptible. Significance Code: p<0.05*, p<0.01**

Individual Variation in Escape and Upright Coping Behavior on Day 1 Maps onto Future Reactivity

Previous work from our lab had identified that the use of escape on Day 1 of social Defeat was predictive of Resilient or Susceptible outcomes (Murra et al, 2022, and see Chapter 2). Therefore, we observed these coping strategies in the FosTRAP2 group and asked whether they expressed a similar behavioral pattern on Day 1. Future Resilient and Susceptible mice engaged in similar amounts of fights and spent similar amounts of time engaged in the fight on Day 1 of Defeat (**Fig. 3.10A.i,ii;** p>0.05, Mann-Whitney U=41). In contrast to our previous findings, there was no correlation between the ratio of number of fights and SI Ratio (data not shown; r=0.2384, p>0.05). However, we were able to replicate our escape behavior findings in this FosTRAP2 cohort. Interestingly, social interaction ratios at the end of the study had a

significant negative Pearson correlation with the number of Escapes (**Fig. 3.10B.ii;** r=-0.609, p= 0.003) and a significant positive Pearson correlation with the number of Upright Freezes (**Fig 3.10C.ii;** r=0.444, p=0.034). Moreover, among the behaviors exhibited during Day 1 behavioral response to social threat, Escape and Upright freezing behaviors emerged as significantly correlated (r=-0.620, *p*=0.002), reflecting two distinct coping styles. This indicates that animals who are more likely to develop future social avoidance are engaging in more escape behavior and less upright freeze behaviors on Day 1.

Periventricular Zone Activity Correlates with Future SI Ratios and Prelimbic Area Activation Predicts Upright Freezing Behavior

As the FosTRAP+ activation patterns are specific to Day 1, we next asked if activation in a singular brain region was correlated to 1) future social interaction ratios, and 2) escape or upright freezing behaviors.

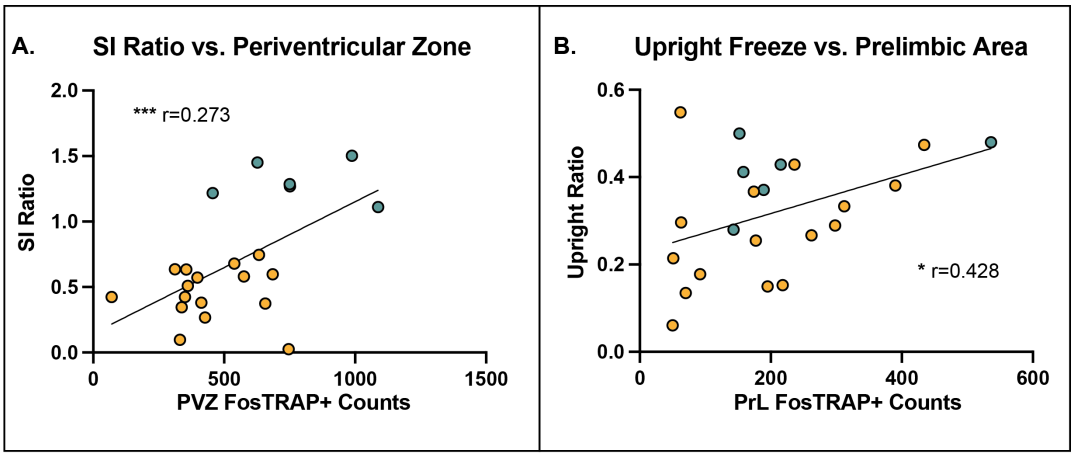

Figure 3.11 Correlates of Brain Activity and Coping Style or Social Reactivity.
A. SI Ratio has a significant positive correlation with number of FosTRAP+ counts within the Periventricular Zone (PVZ) B. Day 1 Upright Freeze Behavior. B. Upright Freezing Behavior has a significant positive correlation with number of FosTRAP+ counts within the Prelimbic Area (PrL). Colors: Blue= future Resilient, Yellow= Future Susceptible. Significance Code: p<0.05*, p<0.01**

In assessing a correlation between a continuum of Social Interaction Ratio and FosTRAP+ counts in each brain region, the periventricular zone was the only region to have a significant positive correlation. An increase in Day 1 activity in the periventricular zone was highly correlated with increased Social Interaction (r=0.273, p=0.007). In addition, while Escape behavior had no overall significant correlations with any individual brain regions, Upright Freezing behavior had a significant positive relationship with FosTRAP positive neurons within the Prelimbic area (r=0.428, p=0.047).

Neural Activity Patterns Predict Escape and Upright Freezing Behaviors

Next, we wanted to know Day 1 FosTRAP Activation was predictive of the type of coping style used during the Day 1 defeat encounter. To that end, we ran a binomial regression analysis with the FosTRAP+ counts as the independent variable and the number of escapes or upright freezes as the dependent variable. We found that there 9 total brain regions that predicted escape behavior on Day 1 of defeat **(**see **Table 3.11)**. In addition, we found one brain region, the Prelimbic Area, that was predictive of upright freezing behavior (**Table 3.12).**

Table 3.11 Brain Regions Predicting Overall Escape Behavior

Region	Estimate	StdError	Z.value	P.value	Adj.P.val	↑Activity Associated With
MD	0.0014	0.00044	3.2827	0.0010	0.02180	↑Escape
BLA	0.00190	0.00059	3.2402	0.0012	0.02180	↑Escape
LA	0.0034	0.0012	2.9418	0.0033	0.0344	↑Escape
Co	0.0012	0.0004	2.8261	0.0046	0.0344	↑Escape
OA	-0.0033	0.0009	-3.6791	0.0002	0.01312	↓Escape
PrL	-0.0028	0.0008	-3.5681	0.0003	0.01312	↓Escape
PVZ	-0.0012	0.0004	-3.0344	0.0024	0.03439	↓Escape
MRn	-0.0165	0.0057	-2.9010	0.0037	0.0344	↓Escape
BNST	-0.0016	0.0006	-2.8320	0.0046	0.0344	↓Escape

Green Indicates an increase in escape behavior and red indicates a decrease in escape behavior

Table 3.12 Brain Regions Predicting Overall Upright Freeze Behavior

Region	Estimate	StdError	Z.value	P.value	Adj.P.val	↑Activity Associated With
PrL	0.0022	0.00063	3.5051	0.0005	0.03332	↑Upright

Green Indicates an increase in upright freezing behavior and red indicates a decrease in upright freezing behavior

BNST and LSNv seed networks were not predictive of specific coping behaviors

As found earlier, the BNST and LSNv were significantly increased in the Defeat condition in comparison to Controls. We did not want to bias the network selection for Future Susceptible or Resilient, and therefore chose the networks that were the most correlated in the overall Defeat condition. Therefore, we combined the counts from the brain regions that were significantly correlated with the BNST or LSNv to ask whether those networks of activations predicted coping behavior. In a linear regression of the BNST and LSNv seed networks, there was no effect on overall escape, upright freezing, or fight behavior, indicating these networks are not predictive to specific coping behaviors (**Table 3.13**; $p>0.05$ all).

Table 3.13 Linear Regression of Network Counts and Coping Behavior

Comparison	Estimate	StdError	Z.value	P.Value
BNSTEscape	0.0000	0.0000	0.1972	0.8437
BNSTUpright	0.0000	0.0000	-0.4011	0.9680
BNSTFight	0.0000	0.0000	-0.3619	0.7174
LSNvEscape	0.0000	0.0000	0.3752	0.7075
LSNvUpright	0.0000	0.0000	-0.2557	0.7982
LSNvFight	0.0000	0.0000	-0.2530	0.8003

3.4 Discussion

Understanding how the brain responds to an initial defeat encounter may prove very useful in elucidating how vulnerability to social stress arises. At baseline, before stress occurs, a limited number of studies have assessed how activity in brain regions selected *a priori* reflect future states of vulnerability (Hultman et al., 2018; Muir et al., 2018). The majority of other studies have focused on neural circuitry or activity patterns that elicit the social avoidance behavior after all 10 days of social defeat (Bagot et al., 2015; Chaudhury et al., 2013; Laine et al., 2017; Muir et al., 2020). In our study, we used novel transgenic mouse technology to ask whether brain-wide neural activity patterns during the initial defeat encounter sets the course for the social outcome. *The current work points to specific brain regions whose level of activation and correlated neural network activity patterns can predict individual variation in coping behavior during the first defeat encounter as well as the eventual Resilient or Susceptible phenotypes.*

Key Findings:

1. Social defeat enhanced the coordination of neural activity across brain regions at a brain-wide level. This was especially evident in animals that would later emerge as resilient.
2. Analyses of correlational neural activity *within each group* revealed the *Lateral Septum* to be a major hub—it was the region with the most significantly correlated other brain regions in both Control and Defeat conditions. Importantly, distinct hubs emerged when focusing on subgroups of mice that would be eventually classified as Resilient or Susceptible. In future Resilient mice, the *hippocampal CA1 region* was the major node. In future susceptible mice, the *basomedial amygdala* was the major node in this

analysis—i.e., these regions were associated with the highest number of significantly correlated brain areas.

3. *Direct comparisons between groups* revealed that the Defeat experience selectively increased activity in the *BNST and LSNv* relative to Control. Using these two areas as seeds for correlational analyses showed that the BNST and LSNv served as key nodes in the coordinate, brain-wide activation of a network of over 50 other brain regions, thereby revealing a highly orchestrated response to social defeat during the first day of exposure.
4. Future Resilient and Susceptible outcomes may be mediated by differences in activity. within the *MHb,* as future Resilient mice showed a selective increase in FosTRAP+ cells in comparison to both Control and future Susceptible mice. The MHb activation in the future resilient group had a distinct, smaller network of activation, as it correlated significantly with both the *lateral septum and several thalamic nuclei*.
5. An increase of activation of the *PVZ* on Day 1 of defeat was significantly correlated with future social interaction ratios, pointing to this region as predictive of susceptibility or resilience.
6. Mice that engaged in greater escape and lower upright freezing behavior on Day 1 of Defeat were more likely to develop future susceptibility.
    a. We found that activity multiple brain regions on Day 1 that predicted the use of escape coping behavior and 1 brain region that predicted upright freezing behavior.

Together, these findings demonstrate that the initial experience with social defeat induces a highly orchestrated brain-wide pattern of neural activation centering on some key anatomical nodes that were revealed by two major approaches- within group and between group

comparisons. Foremost among these regions are the Lateral Septum and the BNST that strongly encoded defeat, the basomedial amygdala that encoded future susceptibility, and the hippocampal CA1 area and medial habenula that encoded future resilience. Moreover, activation in the PVZ on Day 1 correlated positively with future social interaction ratios. In addition, the degree of activation within certain brain regions were predictive of escape or upright freezing behavior, which is in turn predictive of future social outcome. The discussion below frames these findings in the context of what is known about the connectivity and function of these key regions.

3.4.1 FosTRAP: Advantages and limitations

As we consider our findings, it is important to keep in mind the advantages and limitations of the FosTRAP technology. First, the FosTRAP system relies on a tamoxifen-dependent recombinase CreER that is driven by the promoter for the immediate early gene Fos and loxP flanked effector gene, tdTomato (DeNardo et al., 2019). When 4-OHT is present, these active cells undergo recombination and excise the loxP transcriptional stop signal to persistently label active neurons 'tomato' during a specific time window. This technology allows us, for the first time, to capture brain-wide activity in living animals, ascertain their neural response during the first episode of stress, relate that response to concomitant behavior, and leverage this information to predict the divergence in outcome as the chronic stress experience continues and concludes.

However, the 'TRAPing' time window—i.e., the length of time that 4-OHT remains in the body and reaches the brain, can persist for up to 6 hours (Guenthner et al., 2013). Therefore, while the injection was timed for peak Tomato+ labeling following stress, the activity patterns that are captured likely include both the agonistic encounter itself, as well as the psychosocial stress that can persist during the first day of either Defeat or social encounter in the Control

condition. While we can be certain that we are only capturing Day 1 behavior, the sensory/psychosocial elements beyond the initial physical encounter are likely to be also labeled by the FosTRAP system.

Moreover, as is the case with all Fos studies, it is important to note that FosTRAP+ expression may not provide a complete picture of neuronal activity during a given stimulus, as 1) there are some brain regions that do not express Fos/FosTRAP+; and 2) the absence of Fos/FosTRAP+ does not signify a lack of activation (Bullitt, 1990; Cullinan et al., 1995). As such, additional studies with the ArcTRAP transgenic system ('Arc' driven CreERT2) may be necessary to uncover the entire brain activation pattern associated with the initial Defeat encounter (Guenthner et al., 2013). As importantly, Fos/FosTRAP can label both GABAergic and Glutamatergic neurons, and the net effect on the projection area cannot be easily discerned without further dual labeling studies. Thus, there are regions that might be strongly inhibited by the stress conditions and this inhibition is not readily distinguished in the current analyses.

3.4.2 The Majority of Stress-Responsive Brain Regions are Active During Both Social Interactions and Stress Related Behavior, but there are Clear Differences in Coordination of their Activities

In our assessment of 73 brain regions (exploratory) or 27 brain regions (candidate), it appeared that in the majority of brain areas, both Control and Defeated, as well as future Resilient and Susceptible groups exhibited similar levels of activation. This could be due to a number of factors, as the initial experience of being placed in a novel social environment or in the Defeat encounter activates arousal, stress as well as social systems (Martinez et al., 2002; Perkins et al., 2017; Tanimizu et al., 2017; VanElzakker et al., 2008). Only a very small number of brain regions emerged as highly significantly different between groups.

This shared stress response has been previously observed. When assessing differences in c-fos expression between handled Controls (HC) and Syrian hamsters who went through an acute agonistic encounter, Kollack-Walker et al also found that the HC group exhibited activation in numerous brain regions, especially those involved in chemosensory and hypothalamic regions (Kollack-Walker et al., 1997). Thus, activation of these brain areas during the initial encounter may be involved in a general stress and social response.

However, when examining patterns of activation exhibited by each of the groups, as depicted by the Heatmaps (**Fig. 3.4 and 3.5),** it is evident that social defeat triggers a high degree of coordination across multiple brain regions relative to the control condition. In other words, animals who exhibit a strong response to defeat in one area are highly like to exhibit a strong response in many other regions in a highly orchestrated manner. Equally remarkable is that the signature of social defeat is visibly different in the animals that will emerge as resilient vs. susceptible, with the resilient animals showing a remarkably coherent response. This led us to ask which brain regions serve as hubs for these different patterns of integration *within each of these groups.* These regions were used as seeds to ask which other brain regions highly correlated with their level of activation.

In addition, we used stringent statistical criteria to compare levels of FosTRAP activity *between groups.* We found two distinct brain regions that were selectively activated by Defeat (BNST and LSNv), and one brain region that distinguished future Resilient versus Susceptible outcomes (MHb). Based on the unique differential reactivity of these regions, we used them as seeds in our correlational analyses to highlight the differences between the stress groups centering around the regions that best distinguished them.

3.4.3 Within Group Analyses: A Role for the Basomedial Amygdala in Mediating Future Susceptibility

A within group analysis identified distinct hubs in Future Resilient and Susceptible mice. In future Resilient mice, the hippocampal CA1 region emerged as a major node that significantly correlated 22 brain regions that passed FDR-correction. As part of the HPA axis, the CA1 sends GABAergic afferents to the BNST and PVN to mediate the stress response (Herman et al., 2005, 1995). Moreover, activity within the CA1 region has been shown to be differentially attuned to acute versus chronic stressors. In a longitudinal study where local field potentials were recorded within the CA1 region on the first and last day of chronic stress, researchers found that the initial stress experience increased firing in the CA1, while repeated exposure to stress led to decreased firing (Tomar et al., 2021). Without the temporal resolution, we do not have clarity on the specific activation pattern of the CA1, however, it is clearly highly involved in the initial acute stress response of future Resilient mice.

In future Susceptible mice, the BMA emerged as a major node that significantly correlated with 52 brain regions that passed FDR correction. Electrophysiology and optogenetics studies have demonstrated that selective populations within the BMA code aversive or safe environments (Sangha et al., 2013). In addition, recordings within the BMA during controllable and uncontrollable stress revealed that uncontrollable stress leads to higher BMA activity than a controllable stress stimulus (Adhikari et al., 2015). Auditory and visual information about predators is also processed through the BMA, as lesions to the BMA have been shown to reduce freezing behaviors in response to predator pheromone exposure (decreased defensive coping) (Martinez, Carvalho-Netto, Ribeiro-Barbosa, Baldo, & Canteras, 2011). Therefore, the node of

activation within the BMA in future Susceptible mice is possibly associated with threat and anxiety-like states that may set the course for future vulnerability.

3.4.4 Between Group Analyses: The Bed Nucleus of the Stria Terminalis Activation Represents Features of Stress Modulation, Social Interaction, and Anxiety-Related Behaviors

The BNST is a region of high interest in psychiatric disorders, as it is known to be a sexually-dimorphic region that regulates a number of social and memory-related behaviors (Flanigan and Kash, 2020). For example, regulation of fear behavior, social attachment behaviors, aggression-related behaviors, initiation of mating, and arousal have all been shown to be mediated through this highly anatomically connected region (Lebow and Chen, 2016). In our Defeated animals, it appears that activation of the BNST most significantly correlates with several brain regions to which it has direct anatomical connections. Here, we will focus on a select number of regions that a) were significantly correlated with BNST activation; b) are known to have direct or indirect connections with the BNST; and c) are implicated in stress, social, and aggression circuits.

The BNST can limit the stress response and promote social interactions

Interestingly, likely due to the great complexity of this region in terms of subtypes of neurons and their projection, it is difficult to assign a role for the BNST in either mediating the negative aspects of the stress response or in terminating that stress response. First identified in the laboratory of Stanley J. Watson, a neural circuit that is critical in the termination of the stress response arises in the hippocampus, with a glutamatergic projection to the BNST. In turn, BNST GABAergic neurons project directly to the PVN to inhibit the activity of corticotropin releasing factor (CRF) neurons and limit the stress response (Herman et al. 1995; Cullinan et al. 1993). This then places the BNST within the central glucocorticoid negative feedback circuit that tracks

and limits the stress response. Moreover, optogenetic stimulation of GABAergic BNST projections to parvalbumin nucleus accumbens neurons increased social interaction behavior and reduced anxiety-like behavior (Xiao et al., 2021). This role of the BNST may be especially prominent in our Control conditions, as activity in both the PVN and NAc is nominally correlated with the BNST. Thus, when Control animals are placed in a novel social setting the initial stress response (as indexed by activation of the PVN) may be contained and counterbalanced by the rewarding aspects of the social experience and the BNST may play a role in this entire process.

The BNST can mediate the encoding of threat and fear responses:

By contrast, the increased activation of the BNST in the defeat condition may relate to other projections that encode negative aspects of the experience. Under defeat, all 4 sub-regions of the hippocampus (CA1, CA2, CA3, and DG), as well as the PVN are highly correlated with BNST activity. Moreover, in Defeat animals, the BNST is significantly correlated with multiple amygdala regions. The BMA, basolateral amygdala (BLA), central amygdala (CeA), and medial amygdala (MeA) have all been shown to receive direct input from the BNST (Dong et al., 2001a; Jasnow et al., 2004; Markham et al., 2009; Nordman et al., 2020; Walker and Davis, 1997). CRF projections from the BNST to the CeA go on to activate the HPA-axis, which is also modulated upstream through a projection from the BLA to the CeA. The BLA-CeA projection is known to be involved in processing threat-related information (Dong et al., 2001b; LeDoux, 2007). The addition of the hippocampus and amygdala regions in the Defeat condition may represent a sustained stress response that is elicited through the physical and psychosocial stress component.

The BNST is implicated in aggression-related behavior:

Nordman et al., found that the act of winning an agonistic encounter increased synaptic transmission between the MeA to the BNST and ventromedial hypothalamus (VMH) prior to subsequent encounters (Nordman et al., 2020). Indeed, in Defeat animals, the MeA and VMH are highly correlated with the BNST. The initial experience of Defeat therefore may be activating BNST-related circuits that involve the stress response, social cues, and threat-related assessments.

3.4.5 Between Group Analyses: Lateral Septal Nucleus, Ventral Part is Involved in Aggression Related Behaviors

In addition to the BNST, the LSNv selectively increased activation in Defeat animals in comparison to Controls. Similar to the BNST, LS is a neurochemically diverse structure and acts as a relay station between multiple brain regions, including the prefrontal cortex, hippocampus, limbic regions (BLA, MeA, ventral tegmental area (VTA), BNST, and PVN) and transmits information to hypothalamic and thalamic regions to regulate an individual's internal emotional and social state (Menon et al., 2022). As it is a hub between multiple brain regions, it is not surprising that within the Defeat group, LSNv activity is highly significantly correlated with several other areas.

The LSNv plays a role in aggression and other social behaviors:

The classic view of the lateral septum is that it inhibits aggression. Indeed, observations of "septal rage" septal tumors in humans were replicated in a wide range of animal studies (see Menon et al., 2022). Our findings show that LSNv activity is significantly correlated with activity in the VMH and CA2. This is of particular interest, as optogenetic activation of a projection from the CA2 to the LS has been used to disinhibit the VMH and trigger the termination of ongoing attacks during agonistic encounters (Lee et al., 2014; Wong et al., 2016)..

During the agonistic encounter, mice continuously engage and terminate their physical bouts of fight behavior. As we do not have the temporal resolution to capture bouts of fighting vs. non-fighting behavior, the activation of all 3 brain regions quite possibly reflects both states. Therefore, this circuit may be modulating aspects of the aggression behavior in Defeat animals on Day 1. In addition to the CA2, LSNv activity is significantly correlated with activity in the CA1, CA3, and DG. In a resident-intruder paradigm in rats, an elevation of Fos protein expression was found in all 4 subregions of the dorsal and ventral hippocampus (Calfa et al., 2007). When a glucocorticoid antagonist was delivered into the LS prior to the final stress experience, the number of Fos+ cells throughout the hippocampus was reduced, in addition to a reduction in social avoidance behavior. This suggests that the LS mediates social avoidance via a glucocorticoid-sensitive mechanism (Calfa et al., 2007). It is therefore notable that in both the Control and Defeat conditions, the LS was the most highly significantly correlated region, as the modulation of social and emotional states appears to be highly dependent on the LS network.

3.4.6 Between Group Analyses: Medial Habenula is Selectively Activated in Future Resilient Mice

In recent years, the habenula has been implicated in a number of psychiatric disorders, such as depression, ADHD, and schizophrenia (Hikosaka, 2010; Lee and Goto, 2011). Unlike the lateral habenula, which has been extensively implicated in mediating reward and depression-like behavior, the medial habenula (MHb) and its role in these behaviors is less known. Lesions and optogenetic studies in the MHb have implicated it in exercise motivation, hedonic state, and reinforcement learning (Hsu et al., 2016, 2014).

Within the Resilient animals, a small network appeared to be correlated with the MHb. The posterior complex of the thalamus, a region involved in tactile-sensory integration was the

most correlated, indicating that there may be some social-sensory related circuit relayed or activated in coordination with the MHb (Burton and Jones, 1976; Casas-Torremocha et al., 2017). This network also comprises the centrolateral nucleus (CL) of the thalamus, which has a known connection with the MHb, and has been associated with attentional functions, as lesions to the CL cause metabolic cortical depression (Raos et al., 1995). Moreover, the lateraldorsal nucleus of the thalamus (LD), a relay region that provides inputs to higher order limbic-cortical areas was highly correlated with the MHb seed (Bezdudnaya and Keller, 2008). In addition to the sensory-related regions, the LSNv, a region described above as being involved in social aggression, was also highly correlated with the MHb. This may seem contradictory at first, as activation of the LS is known to decrease aggression. However, with the temporal window of 4-OHT injection, we may be capturing features of the psychosocial stress component, in which the aggressive encounter is ended, and mice are placed across from the CD1 with no physical interaction possible. Therefore, we may be capturing future Resilient-specific circuits that integrate sensory-related information about the initial aggressive encounter.

3.4.7 Activity within Select Brain Regions Predict Final Social Outcome and Initial Coping Behavior

Greater Activation of the Periventricular Zone (PVZ) is Associated with Future Resilience

We found that individuals who exhibited a higher level of social interaction following stress, and subsequently greater resilience, had a higher number of cell counts within the PVZ on Day1. In our previous work (Murra et al., 2022), we found that there was a significant correlation between corticosterone levels and increased social interaction when mice were placed back in a social stress context (the forced interaction test) following social defeat. We hypothesize that increased PVZ activity during the first social defeat encounter reflects a robust hormonal stress

response that was maintained after repeated defeat. The PVZ consists of both parvocellular and magnocellular neurosecretory cells. Parvocellular cells express corticotropin releasing hormone (CRH), while magnocellular cells express oxytocin and vasopressin (Dudas et al., 2013). Future studies that explore which cell type co-localizes with these PVZ FosTRAP+ cells may reveal how initial activation of these stress or social-related cell-types mediate the final social outcome.

Activity within Multiple Brain Regions Predicts Escape Coping Behavior on Day 1

We found that activity within 9 brain regions was able to predict the use of Escape as a coping behavior on Day 1 of defeat. Specifically, we found that an increase of activity within the mediodorsal thalamus (MD), basolateral amygdala (BLA), lateral amygdala (LA), and cortical amygdala (Co) area was associated with an increase of use of escape. The increase of activity within these amygdala regions indicates that the use of escape behavior may be associated with differences in threat processing. For example, one study found that when using inhibitory DREADDs to silence the BLA in rats during an imminent, yet escapable threat, rats increased in freezing behavior and reduced their escape performance (Terburg et al., 2018). From this study, the Terburg et al. concluded that the BLA was necessary for the switch from defensive coping (freezing) to active coping (escape) behavior. Interestingly, the MD is known to be a hub in a triangular connection between the prefrontal cortex (PFC) and BLA, as the PFC projects to the MD and the MD projects to the BLA, and BLA back to the PFC (Lee and Shin, 2016). As a central feed-forward hub, lesions to the MD have shown deficits in working memory, emotion, and attention (For Review, see Lee and Shin, 2016). Therefore, the activity we see within the MD and high escape again indicates an active process in responding to threat. We also found that an increase of escape behavior correlated with a lower social interaction score, indicating that activity within these predictive brain regions may play a role in future susceptibility. In contrast,

we found that an increase of activity with the orbital area (OA), prelimbic area (PrL), periventricular zone (PVZ), magnocelluar nucleus (MRn), and BNST was associated with a decrease of use of Escape. As stated above, activity within the PVZ was highly correlated to future resilience. We also found that a decrease in escape behavior was associated with future resilience, implicating both this coping behavior and brain activity in future social outcome. Notably, activity within BNST was not only found to be a hub region that was specific to the defeat encounter, but also as a region that is highly predictive of low escape behavior. As stated above, the act of winning a defeat encounter is associated with increased activity within the BNST (Nordman et al., 2020). Therefore, this initial activation of BNST activity may reflect a decrease in active avoidance behavior and an increase in a positive perception of the defeat encounter. This initial 'winning' scenario, as indicated by BNST activity, may therefore map on to future resilience, as a decrease of escape is correlated with an increase of SI ratio. Overall, the brain regions that are highly predictive of escape behavior are not only known to have anatomical connections between each other, but also are known to mediate aspects of threat assessment and active response. From this work, we can begin to envision creating a predictive model in a novel cohort that combines Day 1 brain activity patterns and escape behavior to predict future social outcome.

Activity within the Prelimbic Area Predicts Escape and Upright Freezing Coping Behavior on Day 1

We found that an increase of FosTRAP+ cells in the prelimbic (PrL) area predicted greater upright freeze and lower escape coping behavior on Day 1 of defeat. Freezing responses to innate threats (such as an approach of a predator) are mediated by lateral, central, and medial amygdala inputs to the periaqueductal gray to elicit the motor response (LeDoux and Daw,

2018). One region where threat processing initially occurs is the PrL cortex. Input from PrL region of the medial prefrontal cortex has been shown to increase amygdala activity in response to stress (Marcus et al., 2020). Therefore, the relationship between the increase in FosTRAP+ cells within the PrL and greater upright freezing may reflect an increase in perceived threat potential within these individuals. Similar to the predictive model, we found that the use of upright freeze was negatively correlated with escape behavior, indicating that these two coping styles are inversely related. The transition from avoiding detection (freezing) and active avoidance (escape) is mediated through opposing neural circuits that converge on the amygdala, suggesting that the shift from reaction to action during the initial stress experience represents differentially mediated coping behaviors that become strengthened through neural responses as the animal learns or habituates to the stress experience (LeDoux, 2016). Importantly, a higher rate of upright freezing on Day 1 was significantly correlated with an increase in social interaction ratio after stress, indicating that future resilient mice likely begin the social defeat paradigm with a tendency to use a coping strategy that has been labeled as "attentive immobility" (Bracha, 2004; Kozlowska et al., 2015; Marks, 1987). In our previous work, we found that these behaviors shift, whereby escape behavior increases by Day 10 in both groups, suggesting that there is a learning response that occurs within Resilient mice as the defeat continues (Murra et al., 2020). On the other hand, susceptible mice are more likely to escape than freeze on Day 1. The decrease in PrL activity in future susceptible mice indicates that the generalized fear response may not be present within these animals early on, allowing them to exhibit a more active avoidance coping behavior during the first encounter.

3.4.8 Other Components of the Defeat and Resilience Circuitry

While this discussion has focused on the areas that emerged as the most significant in using either the within or between group approaches, there is clearly a broad range of brain regions that participate in the defeat and the resilience networks that are either nominally or significant in their own right. Each of these has its own anatomical connectivity and functional role in mediating the observed behaviors and their immediate and long-term consequences. For example, both the Defeat BNST and LSNv networks showed a significant correlation with the anterior cingulate cortex (ACC). In Control animals, the BNST has a nominal significant correlation with the ACC. Both human and rodent studies have shown that the ACC is involved in empathy, pro-social behaviors, reward, and social attention (Apps et al., 2016; Devinsky et al., 1995; Schneider et al., 2020). Thus, we may be picking up a feature of social motivation and modulation that reflects different internal emotional states in both the Control and Defeat conditions.

Overall, the FosTRAP approach has captured a rich network of activation that appears to include sensory, emotional, motivational, and compensatory mechanisms of coping with a social encounter. We have revealed similarities as well as some clear differences induced by social defeat and pointed to areas that predictive of coping strategy. Notably, that Day 1 brain activity patterns are highly predictive of escape behavior, which is also highly predictive of future susceptibility to CSDS.

3.4.9 Future Directions

While this body of work has mapped the brain regions that are affected by the initial stress or social experience, it is clear that each of the regions, especially the 5 that emerged as pivotal (BNST, LSNv, and MHb as well as BMA and CA1) are individually complex and likely

play multiple roles in mediating the stress response. As in the case with the BNST, LSNv and BMA, a large portion of brain-wide networks (50+ regions) are engaged with these hubs, implicating their role in mediating several aspects of the coping response (aggression or escape) and future susceptibility or resilience. These regions are also highly diverse and densely anatomically innervated, as activation of certain cellular populations are attuned to different features of the stress or social response. Future studies that identify the biochemical identity of these FosTRAP+ neurons (i.e., are they excitatory or inhibitory? Do they express a specific neuropeptide or other distinctive marker? Do they project to a particular element of the circuitry?) are needed to further characterize these neural circuits. Moreover, follow up studies that selectively activate or inhibit these targeted seeds are needed to determine their role in encoding not only coping strategies, such as escape, but also the propensity for future susceptibility or resilience.

While we assessed differences in brain-wide activation patterns that were present during the initial Defeat encounter, understanding how these patterns sustain or change as a result of going through the entire chronic stress experience is an important factor in uncovering the dynamic nature of the stress response. Mice that are exposed to acute social defeat (<5 days) do not show differences in social reactivity in the social interaction task, therefore the experience of chronic defeat is critical in manifesting the individual differences in social behavior (Bagot et al., 2015; Kudryavtseva, 1994). In Syrian hamsters, repeated compared to acute exposure of social Defeat resulted in sustained, reduced, or increased levels of c-fos mRNA within select brain regions, a feature of differential brain-wide adaptations (Kollack-Walker et al., 1999). At the neuroendocrine level, both the acute and chronic stress experience elicited the same corticosterone (CORT) response; subordinate males showed an acute CORT response in

comparison to dominant and control males. This overall suggests that repeated exposure to stress may be eliciting a brain-wide habituation response. Future studies that combine the FosTRAP+ system during the initial defeat encounter and, within those same animals, defeat exposure timed for Fos protein at the conclusion of CSDS are needed to understand whether Resilient and Susceptible mice show potential differences in habituation or changes in neural circuits that occur as a result of the chronic stress experience.

3.4.10 Conclusion

For the first time, our study has identified brain regions that are selective to the initial experience of Defeat, as well as Resilient or Susceptible outcomes. In addition, this work has identified networks of brain regions that correlated highly with seed regions we have identified and revealed brain-wide activation patterns. Activation within specific brain regions predict individual differences in coping with the initial stress experience, as well as the future development of vulnerability. Therefore, we can conclude that there are brain-wide neural signatures that are present during the initial defeat encounter that map onto the stress experience that are distinct from the social experience and may reflect future Susceptible and Resilient states.

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Chapter 4 Discussion

As discussed throughout the preceding chapters of this dissertation, chronic, stressful social interactions can lead to a wide variety of outcomes, ranging from adaptive to maladaptive, and are highly dependent on individual variation in the stress response. Despite much research, it is unclear how the initial stress experience may be mediating the differences in social reactivity following stress. In order to better assess how these vulnerability or resilience factors intersect, we used a mouse model of individual differences, chronic social defeat stress (CSDS). While this physical and psychosocial 10-day stressor is delivered to a group of genetically inbred mice, a divergence of social reactivity occurs following stress: ~60% of mice become socially avoidant (susceptible) and 30% become resilient (socially interactive) (Golden, Covington, Berton, & Russo, 2011). Given that mice in these studies share a common genetic background, much of the individual variation in stress response can be attributed to developmental or environmental variables.

We hypothesized that behavioral coping strategies that mice exhibit during the first stress experience would predict whether resilient or susceptible social reactivities arose following repeated social stress. In addition, neural circuits involved in coping responses, stress, and social behavior tend to converge on overlapping but distinct brain regions (See **Chapter 1, Fig. 1.6)**. We thus hypothesized that differences in brain-wide activity patterns present during the initial stress experience would reflect specific features of coping responses and can be used to predict the type of social reactivity that arises following chronic social stress.

In using the CSDS model, we were interested in the question: How do behavioral responses and neural circuits that emerge early on during the stress experience shape individual differences

to vulnerability? To approach this larger question, we endeavored to answer two overall questions:

1. ***When and how do individual differences in reactivity to social stress arise?***
2. ***Are there distinct patterns of neural network activation during the initial social stress experience that set the course for eventual susceptibility or resilience to chronic social defeat****?*

Hypothesis: Our overarching hypothesis was that brain-wide activation patterns and behavioral coping styles that emerge during the initial defeat encounter map onto future resilience or susceptibility.

4.1 Summary of Findings

In **Chapter 2**, our behavioral and neuroendocrine characterization of resilient and susceptible animals at multiple time points before, during, and following stress revealed novel findings showing that individual variation in sociability was only apparent in behavioral measures once the stress began, that the response to chronic stress was highly dynamic, and that susceptibility to social stress was highly specific to the social stress context.

A main finding of our behavioral analysis is that the *initial* coping response to stress is a predictive risk factor in future development of vulnerability. In particular, the active coping response of escaping from an approaching aggressor indicated a greater likelihood of developing susceptibility, while the active response of engaging in the fight was correlated with future resilience. Both groups engaged in similar amounts of passive coping (freezing), indicating that **differences in active avoidance behavior mediates future stress reactivity.**

Our longitudinal analysis of multiple indices of stress responsiveness across the course of the chronic social stress experience revealed the following:

- Before stress
    - The degree of social interaction before stress was not predictive of social response following stress.
    - Basal corticosterone had no predictive value for social reactivity to CSDS.
- During Stress
    - **Coping style during the first defeat encounter was a predictive variable in the emergence of differences in sociability: higher rates of escape behavior on Day 1 indicate greater likelihood of a subsequent classification as susceptible in the social interaction test.**
- Following Stress
    - Social avoidance behavior was not generalizable to other traditional measures of affective behavior.
    - Social avoidance was associated with sensitivity to physical pain, significant weight loss, and a lower CORT expression when placed back in the social stress context.
    - **Social avoidance is social context-specific**, as susceptible mice do not avoid non-aggressive strains or their conspecifics.

In **Chapter 3**, we used novel mouse technology (FosTRAP) to capture brain-wide neuronal ensembles that were activated during the initial social defeat encounter and assessed whether specific regions or networks of regions could accurately predict Day 1 coping behavior and subsequent social reactivity following stress. Overall, we found:

- The experience of defeat overall increased network coordination (correlation between brain regions), largely driven by future Resilient animals.

- An exploratory within group network analysis revealed differential nodes within future Resilient and Susceptible mice. In Resilient mice, the hippocampal CA1 region was identified as a highly coordinated hub. In Susceptible mice, the basomedial amygdala (BMA) was identified as a highly coordinated hub, with a notable number of regions whose neural response correlates with the magnitude of the BMA response.
    - BMA network-related activity may be predictive of future Susceptibility.
- When directly comparing the Social Defeat Group to the Control Group, specific regional differences of activation in the bed nucleus of the stria terminalis (BNST) and lateral septal nucleus, ventral part (LSNv) were associated with the defeat experience.
    - The increased activation of these 2 regions in social defeat was accompanied with highly correlated changes in brain-wide networks, indicating that there is a unique signature to the initial defeat experience.
- Regional activation differences in the MHb were associated with Future Resilience
    - This seed region contained a unique activation network within Future Resilient mice that significantly correlated with brain regions associated with sensory-motor and aggression-related behaviors.
- Mice who engage in an increase of escape and decrease of upright freezing behavior on Day 1 of defeat were more likely to develop social avoidance (susceptibility).
    - Activation within selective brain regions predicted Escape or Upright Freezing Behavior
- Activation of the Periventricular Zone (PVZ) on Day 1 correlated with an increase of social interaction ratio following stress

Main Discovery: Individual variation in coping response during the initial stress encounter represents a predictive element in future social reactivity following stress. Importantly, brain-wide networks of activation associated with the initial defeat encounter represent elements of the experience of psychosocial/physical stress, as well as future social reactivity. The initial activation of the PVZ was able to predict future SI ratio. In addition, activation patterns within certain brain regions were able to predict the use of escape or upright freezing coping behaviors on Day 1, and therefore the overall the propensity to develop vulnerability to social stress.

4.2 Limitations of Our Work

The N's in our study are not consistently powered to robustly detect some of the differences across the various dimensions we have examined. While our coping behaviors study (**Chapter 2**) was a combination of 4 cohorts of mice, some of the behavioral and neuroendocrine characterizations occurred in cohorts in which there were ~20 mice, ~60% of which developed susceptibility. The lack of findings from our baseline social behaviors, as well as baseline neuroendocrine studies should be repeated in order to assess whether these elements are truly non-predictive. In addition, our FosTRAP study yielded a small number of resilient mice (N=6). Remarkably, these resilient mice exhibited very consistent and strong effects as can be visualized by the heatmaps. Nevertheless, when characterizing neuronal activity patterns over 73 brain regions and correcting for multiple comparisons, we are likely missing some features of meaningful activation patterns. By contrast, the number of susceptible animals was larger (N=16), which likely contributed to our ability to uncover the strong relationship between escape behavior and its neural correlates. Overall, our findings still indicate that defeat-specific brain-wide circuits, as well as coping responses during the initial defeat encounter represent predictive elements of future vulnerability. Moreover, the networks we have identified are consistent with what is known

in the literature about the nodal brain regions, their connections and their role in social interaction, aggression, and stress behaviors.

We also noted in **Chapter 3**, the power as well as the limitations of the FosTRAP system. It is important to recall that with FosTRAP, we are capturing neuronal activation over a longer time window (up to 6 hours) relative to class c-Fos studies. While 4-OHT was injected for peak FosTRAP+ labeling following stress, the activity patterns that were captured most likely include both the 5-minute agonistic encounter, as well as the psychosocial stress that ensues following defeat on Day 1 (Guenthner, Miyamichi, Yang, Heller, & Luo, 2013).

In our network analysis, our models were based on correlations between seed regions and all brain regions identified. While we picked up brain regions that have direct anatomical connections between our seed regions and the regions that were highly correlated, brain regions that are not known to have functional connectivity with our seeds also appeared. This is likely to second order activation but would require extensive anatomical studies to characterize appropriately.

Neuropsychiatric disorders tend to exhibit sex-specific differences in symptomology and rates of expression (Dohrenwend & Dohrenwend, 1976). A major limitation of our work, and of this model, is that CSDS is only conducted in male mice. While recent studies have begun to assess the effects of social defeat in females, applying this model to female mice has been challenge, as the experimental conditions that provoke naturalistic territorial conditions do not occur in females (Harris et al., 2018; Logan, 2019; Takahashi et al., 2017; van Doeselaar et al., 2021). The existing social defeat literature has therefore largely focused on male mice (Miczek, Maxson, Fish, & Faccidomo, 2001), as do the current experiments. Thus, any interpretations of the current results are limited to male subjects and may not apply to the factors involved in social stress processing

in females. A careful examination of how sex-specific coping behaviors contribute to the development of stress vulnerability is critical for our understanding of underlying risk factors for stress-related disorders in humans (Lyons, Buckmaster, & Schatzberg, 2018; Senst, Baimoukhametova, Sterley, & Bains, 2016; Varholick et al., 2019).

In spite of these limitations, our work has provided several key insights into the development of susceptibility. It is also the first study to focus on the initial stress experience of social defeat and define the key elements during that initial session that predict social outcome.

4.3 Expanding Upon the Chronic Social Defeat Stress Model

Below, I will further discuss the nuances behind the CSDS model. In particular, I will focus on expanding upon the traditional classification of resilient and susceptible outcomes as either adaptive or maladaptive, as well as the costs and benefits associated with these sociability outcomes. Next, I will discuss re-thinking the categorization of the CSDS model as an animal model of stress and further expand on what this model can tell us about stress and social-related disorders. Finally, I will conclude by discussing limitations, remaining gaps, and future directions.

4.4 How Adaptive or Maladaptive is Susceptibility to CSDS?

4.4.1 Active Avoidance to Social Stress is Not Necessarily Maladaptive

The variation in individual differences that arise from experiencing the same, chronic stress can be seen to manifest at the level of cognitive and behavioral coping responses. If the stress is too overwhelming and demanding to the individual, it may be reflective of current or future states of vulnerability. In humans, behavioral flexibility in coping response can be seen as a feature of resilience, as the ability to reframe or reason and exercise behavioral control mechanisms during stress tends to lead to positive health outcomes (Russo, Murrough, Han, Charney, & Nestler,

2012). In contrast, poor emotional and behavioral control, characterized by avoidance or suppression of chronic, stressful events tends to lead to maladaptive health behaviors, such as neuroticism and susceptibility to affective disorders (Bienvenu, Hettema, Neale, Prescott, & Kendler, 2007).

Indeed, we found that the *initial* coping response to stress is a predictive risk factor in future development of vulnerability. In particular, the active coping response of escaping from an approaching aggressor indicated a greater likelihood of developing susceptibility, while the active response of engaging in the fight was correlated with future resilience as classically defined by the CSDS model. In assessing multiple cohorts, both groups engaged in similar amounts of passive coping (freezing), indicating that overall, active coping responses mediate future stress reactivity.

Unlike the freezing response, escape is a defensive behavior that an animal engages in when experiencing a looming threat and perceives that there is a 'shelter' location from the threat (De Franceschi, Vivattanasarn, Saleem, & Solomon, 2016). Moreover, this coping strategy tends to become more utilized as the animal memorizes the spatial environment (Vale, Evans, & Branco, 2017). By the final (day 10) of stress, resilient and susceptible mice both shift to similar coping styles, with an overall decrease in fight engagements and an overall increase in escape behaviors. While generally active engagement vs. active avoidance is classified as adaptive or maladaptive, placing them in the context of the stress adds a layer of complexity to this simplistic binary classification. During aggressive encounters, it appears that the animals learn that 'escaping' or actively avoiding by Day 10 prevents the CD1 aggressor from initiating vicious attacks. Thus, this avoidance behavior is adaptive to the animal, as there is a high metabolic cost in constant fight engagements. Therefore, active avoidance in the context of social defeat is not necessarily maladaptive and may represent a protective behavioral strategy in the face of aggression.

4.4.2 Understanding When and Where These Coping Behaviors Arise May Paint a More Cohesive Picture as to the Adaptive Value of the Response

Home cage (HC) social rank has also been shown to be a predictive factor in resilient or susceptible outcomes: HC dominant mice are more likely to become susceptible, while HC subordinate mice are more likely to become resilient (Larrieu et al., 2017). While this might seem counterintuitive at first, the authors speculate that this is likely because the threat to social status is particularly threatening to dominant males, as their social status is challenged the most during defeat encounters (Larrieu et al., 2017). To establish social hierarchy in the HC, mice go through a series of agonistic encounters (Horii et al., 2017; Williamson, Lee, & Curley, 2016). Thus, the type of coping response that a mouse engages in during day 1 of social defeat stress might also be a combination of learned threat and coping responses that were utilized in the HC. This learned response may also reflect neural circuitry associated with coping responses, as certain circuits become strengthened or weakened depending on whether an individual perceives 'winning' or 'losing' the agonistic encounter (Kollack-Walker, Don, Watson, & Akil, 1999; Nordman et al., 2020; Sinha, Lacadie, Constable, & Seo, 2016). Experience shapes behavior; understanding the mechanisms behind how an initial stress exposure sets an individual down a particular path might be a result of a lifetime accumulation of events. Therefore, the circuitry that is apparent on Day 1 may be previously primed by the mice's HC environmental context. HC dominant males may have less experience in being overpowered, and thus may engage in escape coping behavior early on due to their change in expectation during the physical confrontations. Future studies that retroactively assess HC coping behaviors in establishing social rank may provide an additional dimension to predicting resilient and susceptible outcomes prior to intense social defeat.

4.4.3 Neural Circuits Involved in the Initial Stress Response May Help Further Classify the Type of Vulnerability that Resilient or Susceptible Animals Exhibit

We found that the *initial* experience of defeat selectively activates the BNST and LSNv. These two brain regions were associated with overlapping circuitry that indicated a brain-wide defeat response. Research in rodents, non-human primates, and humans has indicated that the BNST plays an important role in the manifestation of anxiety. As a highly complex, cellularly diverse, and anatomically connected region, the BNST integrates many types of information involving social and memory-related behaviors (Flanigan & Kash, 2020). Of particular interest, the BNST is involved in hypervigilance, as it has been as a critical region in responding to diffuse, ambiguous, or unpredictable threats (Goode, Ressler, Acca, Miles, & Maren, 2019; Grupe & Nitschke, 2013). In an fMRI study of health controls, participants were shown a series of aversive versus neutral anticipatory cues. Activation of the BNST, prefrontal cortex, anterior cingulate cortex, and periaqueductal gray appeared only in anticipation of the threatful stimulus. In our study, defeated animals BNST activity was highly correlated with the anterior cingulate cortex and periaqueductal gray, indicating that this 'anticipatory threat' circuit is maintained in both rodent and human neural activity patterns (Herrmann et al., 2016). In another fMRI study, healthy participants with individual variation in trait anxiety were placed in an environmental threat-monitoring task. Individuals who had greater trait anxiety had an exaggerated response in tracking threat proximity, which was correlated to increased BNST recruitment. During the initial social defeat encounter, increased vigilance, or hypervigilance is required in comparison to experiencing only the social aspects of being placed across from another conspecific (initial control rotation experience). Therefore, this increased BNST recruitment seen in defeated mice may indicate an increased hypervigilance that is required during the agonistic encounter.

In humans, a symptom of experiencing SAD or social avoidance behavior is anxiety associated with the anticipation of the next social or public encounter (Hofmann, 2007). The worry and fear about a potential social judgment is an inherently unpredictable situation, as the individual may ruminate on past experiences as an example of what *could* but has not yet happened in the future (Kocovski, Endler, Rector, & Flett, 2005). Clauss et al. used an fMRI task to measure neural responses to unpredictable and predictable cues and fearful or neutral images in individuals that ranged from high to low social anxiety. When presented with an unpredictable cue, individuals who had higher social anxiety also had lower BNST-amygdala connectivity. In contrast, when presented with an unpredictable fearful image, those with increased social anxiety showed an increased connectivity between the BNST and amygdala, as well as the ventromedial PFC (vmPFC). Thus, in socially anxious individuals, activation of the BNST and its corollary network was dependent on threatening visual context cues that are most likely shaped by previous experiences (Clauss, Avery, Benningfield, & Blackford, 2019). Therefore, the BNST is a critical node in anticipating unpredictable threat and its selective activation in the defeat condition may represent features that are present in human social anxiety disorders.

In both humans and rodent species, the lateral septum (LS) is known to be involved in arousal and social behaviors (Clemens, Wang, & Brecht, 2020; Menon, Süß, Oliveira, Neumann, & Bludau, 2022). The role of the oxytocin (OT) system in the lateral septum has been implicated in mediating social fear (Chen, Nishitani, Haroon, Smith, & Rilling, 2020; Menon et al., 2018). As mentioned in the introduction, the OTXR gene has been used as a candidate gene approach in mediating human social behavior. However, a meta-analysis of 2 promising OT single nucleotide polymorphisms revealed no meaningful effect on human social behavior (Bakermans-Kranenburg & van Ijzendoorn, 2014). Despite this, the OT system within the LS may be a feature of a specific

circuit of activation in mediating behavior. In a rodent study of social fear conditioning, infusion of central oxytocin (OTX), but not arginine vasopressin, into the lateral septum prior to social fear extinction training abolished any social fear expression (Zoicas, Slattery, & Neumann, 2014). In a human fMRI study, when intranasal OT was delivered before a social interaction task, there appeared to be widespread increases in functional connectivity in response to positive social interactions. The researchers identified the LS and NAc as hubs for this OT effect on social behaviors and functional connectivity (Rilling, Chen, Chen, & Haroon, 2018). As such, it appears that the molecular character and activation of the LS plays a role in mediating social behaviors. While the studies above suggest that LS activation mediate positive social outcome, we do not have specificity on the type of neurons that are being activated within the LS to mediate these behaviors. In addition, lesions to the LS have been shown to induce 'septal rage', therefore, activation of this brain region may also represent the termination of the aggression-like behavior during the psychosocial component (Wong et al., 2016). Overall, we can state that the initial defeat encounter is picking up elements of social behaviors within the LS, but further studies that identify what types of neurons are being activated are needed to understand the role of this hub.

We found that when assessing the continuum of the social interaction ratio and FosTRAP+ counts within brain regions, the PVZ emerged as a significantly correlated region. An increase in PVZ activity was correlated with an increase of future SI ratios, indicating that its activation may be critical in eliciting a future Resilient outcome. The PVZ contains a diversity of neurosecretory cell-types, including vasopressin, oxytocin, corticotropin releasing hormone, thyrotropin-releasing hormone, and somatostatin (Biag et al., 2012). Within the CSDS literature, previous work has found that acute social defeat enhances corticotrophin-releasing factor mRNA, while chronic social defeat drives an increase in arginine vasopressin mRNA (Keeney et al., 2006). Therefore,

future Resilience mice may be defined by a correct initial mounting of the stress response. This can be further seen by the fact that the highest rank within-group hub in Resilient mice was the hippocampal CA1, a critical node in eliciting the acute stress response (Tomar et al., 2021). In **Chapter 2**, we found that after defeat, when mice were placed back in the social stress context, Resilient mice displayed an increase in CORT response. While PVZ activity was assessed on Day 1, this heightened CORT response following stress may reflect an initial stress-response that does not habituate in future Resilient mice. In addition, in **Chapter 3**, an increase of upright freezing was correlated with a decrease of use of escape as a coping strategy, two behaviors that are known to be differentially mediated (LeDoux and Daw, 2018). An increase of prelimbic area activity was shown to be highly predictive of increased upright freezing and decreased escape behavior. While an escape response is said to be used when an animal is actively engaged in threat and represents a goal-directed response, upright freezing is a feature of attentive immobility, indicating that the animal is attempting to decrease their likelihood of detection (Kozlowska et al., 2015; LeDoux and Daw, 2018). Activity within the PrL is known to mediate selective attention towards relevant stimuli (Broschard et al., 2021; Sharpe and Killcross, 2014; Williams et al., 1999) .Taken together, future Resilient animals are *initially* defined by differences in their level of threat assessment, as an increase in stress-specific circuitry to threat is a feature in these animals. Therefore, on Day 1, future resilient mice shift towards a reactive, rather than active, coping behavior.

Moreover, we found that the Mhb was selectively activated in future Resilient mice compared to future Susceptible. While the lateral habenula has been extensively characterized in mediating depression-like phenotypes in rodents and neuropsychiatric disorders in humans, the role of the MHb is less known (Xu et al., 2018). In a rodent study that investigated the role of the MHb in the expression of anhedonia-like behavior following chronic mild unpredictable stress, the

researchers found that lesions to the MHb reversed the anhedonia-like behavior that was observed in the sucrose preference test following stress (Xu et al., 2018). In our study, the future Resilient mice showed increased activation in the MHb compared to future Susceptible mice. This may at first seem contradictory, as the above findings suggest that activity within the MHb is required to elicit depression-like behavior. However, if we take for example the social hierarchy findings in Larrieu et al. this initial defeat exposure experience may reflect an acute depression-like response. Animals who are lower in dominance status within their home cage, once entering the social defeat paradigm, are more likely to develop Resilience (Larrieu et al., 2017). This first stress response may then be reflective of a history of 'losing' encounters. Further research is needed to investigate the role of the MHb in mediating coping and future social outcome.

Furthermore, in our analysis of within group highly coordinated seeds, we found that the basomedial amygdala (BMA) in future Susceptible animals represented within-group hubs that correlated the significantly most with other brain regions. In general, the amygdaloid complex has been associated with many parts of physiological, behavioral, and neuroendocrine responses to fear (J. LeDoux, 2007; J. E. LeDoux, Iwata, Cicchetti, & Reis, 1988). Each of the sub-nuclei of the amygdala have been shown to participate in different aspects of the fear-response system, such as threat detection, fear conditioning, and heightened anxiety states. Separate neuronal populations within the BMA have been shown to encode safe versus aversive contexts, indicating that it is a highly diverse region associated with fine-tuning the threat response (Adhikari et al., 2015). In addition, BMA activation has been shown to decrease fear-related freezing through activation of top-down vmPFC→BMA projections (Adhikari et al., 2015). However, in our study, despite increased network activation of the BMA in future Susceptible mice, both future Resilient and Susceptible mice had similar rates of freezing responses during the initial stress exposure. As

mentioned in **Chapter 3,** BMA activity has also been shown to be increased in response to contextual predator-odor presentations (Martinez, Carvalho-Netto, Ribeiro-Barbosa, Baldo, & Canteras, 2011). Therefore, the role of the BMA within future Susceptible mice may be integrating information about the aggression-related context to mediate differences in threat detection and use of certain coping behaviors.

Moreover, we found that activity in amygdala regions (lateral amygdala (LA), BLA, and cortical amygdala (Co)) was predictive of escape behavior. In both **Chapter 2** and **Chapter 3**, we found that a greater use of escape behavior was predictive of future susceptibility. As mentioned in **Chapter 3**, impairments to the BLA are associated with a decrease in active avoidance (escape behavior) and an increase with attentional immobility (freezing behavior) (Terburg et al., 2018). Taken together, on Day 1, susceptible mice are defined by their ability to threat discriminate early on. This threat discrimination continues on post-stress, as we found in **Chapter** 2, that susceptible mice are able to distinguish between defeat-associated strains and non-threatening strains. Future studies that explore the role of these amygdala regions in escape coping response to the social stress context are needed to implicate its role in predicting vulnerability.

4.4.4 Post-Stress: Costs, Benefits, and the Language of Resilience

Resilience is defined as "the capacity and dynamic process of adaptively overcoming stress and adversity while maintaining normal psychological and physical functioning" (Russo, Murrough, Han, Charney, & Nestler, 2012). While we attribute resilience as a positive adaptation following stress, this definition is often applied generally, without taking into consideration the environmental in which resilience is displayed. Moreover, resilience is oftentimes referred to as a character trait that exists or does not exist within an individual. However, the reality of experiencing chronic stress is not as simple as this binary framing would imply. Our work suggests

that the classification of susceptible and resilient mice needs to be re-evaluated based on the greater context of where and how these behaviors are manifested.

Following stress, the social avoidance phenotype is not generalizable to traditional measures of affective behavior. In the open field test, which measures anxiety-like behavior based on an animal's willingness to explore the center of the arena, both resilient and susceptible mice displayed a decrease of cumulative duration in the center associated with social defeat. This behavior has been shown to be maintained for 39 days following stress, indicating that defeat alone is sufficient to induce a generalized anxiety-like state (Krishnan et al., 2007). In contrast, we and others have shown that the social avoidance behavior is social context-specific, as susceptible mice do not avoid interacting with non-aggressor CD1 strains (Milic, Schmitt, Lutz, & Müller, 2021; Murra et al., 2022). In this context, the ability to threat discriminate in susceptible mice may be more evolutionarily adaptive, as the heightened level of vigilance does not expand to other scenarios. However, we found that susceptible mice still display an increase in pain sensitivity and weight loss as well as a dysregulated stress response in comparison to resilient, indicating that there still is a physiological cost to expressing social avoidance.

These behaviors may be explained by features of generalized anxiety disorder (GAD) and a more specific social anxiety disorder (SAD). While GAD is characterized by persistent and uncontrollable worrying, SAD manifests itself as a fear specific to social situations that is out of proportion to the reality of the social encounter (National Collaborating Centre for Mental Health (UK), 2013). These disorders overlap, as people with SAD may experience a generalized anxiety state where fear and anxiety are triggered most by social situations, or experience a non-generalized SAD, where the fear is associated with a limited range of situations (e.g., public speaking or certain individuals/groups (National Collaborating Centre for Mental Health (UK),

2013). Therefore, susceptible animals may represent a feature of non-generalized SAD, where social avoidance is only apparent in scenarios associated with previous stress experience. Taken together, our results suggest a greater complexity to the binary resilient vs. susceptible labels.

4.4.5 Beyond Defining CSDS as an Animal Model of Depression

In general, neuropsychiatric disorders are heterogeneous in nature. Individuals with complex sets of characteristics are grouped under the same umbrella of an overarching disorder, despite heterogeneity in both the causes and expression of the disorder. Patients are diagnosed with mental health disorders using the Diagnostic and Statistical Manual of Mental Health Disorders, 5th edition (DSM-V), however, this does not come without caveats. Many critics have stated that the categorization of the DSM-V oversimplifies human behavior and increases the tendency for physicians to over diagnose patients due to blurred boundaries between normal vs. disordered states (Aragona, 2009; Kernberg, n.d.; Pickersgill, 2014). Therefore, terms such as "animal models of depression" or other psychiatric disorders may not be the most representative language in how we model aspects of these diseases in laboratory settings.

Animal models of diseases are usually described through the lens of three types of validity: Face, construct, and predictive. Face validity refers to how well a model replicates the disease signs and symptoms in humans; construct validity refers to how well the mechanism used to induce the disease phenotype in animals reflects the disease etiology in humans; and predictive validity refers to how well the model responds to treatments that predict the effects of treatments in humans. CSDS has been said to have face, construct, and predictive validity as a model of depression. However, CSDS and other animal models of psychiatric disorders tend to meet aspects of each of these validities, but not completely. Let us take for example the definition of MDD from

the DSM-V (**Table 4.1).** If 5+ of these symptoms are present for >2-week period, then a patient would be diagnosed with MDD.

Table 4.1 The symptoms of MDD according to DSM-5, modified from (Friedman, Resick, Bryant, & Brewin, 2011)

Diagnostic Category	Symptoms
Negative Mood And Cognitions	• Feels sad, worthless, empty, hopeless (subjective report) • Recurrent thoughts of death or suicidal ideation • Appears tearful or irritable (observed report) • Diminished ability to think, concentrate, or indecisiveness nearly every day (subjective or observed report) • Distress or impairment in social, occupational, or other areas of functioning
Anhedonia	• Diminished interest or pleasure in all, or almost all activities most of the day or everyday
Weight Changes	• Significant weight loss when not dieting or weight gain (>5% of body weight in a month) • Decrease or increase in appetite nearly every day
Alterations in arousal	• Insomnia or hypersomnia nearly everyday • Fatigue or loss of energy nearly everyday • Psychomotor agitation (restlessness)

Under these diagnostic criteria, the social defeat model, in assuming susceptible mice display depression, does not capture the complete face validity of MDD. While we cannot assess the negative mood of animals (i.e., ask the mice "how are you feeling?"), behavioral tasks that assess the affective-like behavior, cognition, and sociability of the animal act as proxies. Traditional measures of despair and anxiety-like behavior, in our work and others, do not map on to the individual variation that is seen in the sociability behavior. The cognitive effects of CSDS can be assessed through the novel object recognition test (NORT), which assesses the amount of time an animal spends with an object that was shown to them in a previous session. While NORT seemed to be impaired overall in defeated mice a week following CSDS, there was no

discriminatory effects between resilient or susceptible populations. However, this cognitive 'impairment' was diminished at 21 days following defeat (Wendelmuth et al., 2020). Moreover, other groups have found mixed results in a measure of anhedonia, the sucrose preference test. While some have found that susceptible animals have less of a preference for sucrose compared to resilient and controls, others have found no differences between the groups (Alves-Dos-Santos, Resende, & Chiavegatto, 2020; Chaudhury et al., 2013; Henriques-Alves & Queiroz, 2015). In addition, any observable differences in sucrose preference were completely diminished 3 weeks following defeat, despite the maintenance of the social avoidance behavior (Krishnan et al., 2007). Moreover, any observable alterations in arousal do not persist long-term. While susceptible animals have shown a decrease in circadian amplitude immediately following stress, this feature also restored after 3 weeks (Krishnan et al., 2007). In addition, locomotor activity does not differ between resilient and susceptible mice either immediately or 3 weeks following stress (Krishnan et al., 2007).

Assessing construct validity in animal models of depression is difficult, mainly due to the lack of findings in genetic contributions in the development of mood disorders. As mentioned in the Introduction Chapter, epidemiological studies found that the genetic contribution in the development of MDD had a heritability of ~37%, indicating that environmental factors contribute a larger amount of the variance (Sullivan, Neale, & Kendler, 2000). In addition, the largest meta-analysis of over a million individuals found that only 87 significant gene variants associated with depression (Howard et al., 2019), likely due to the great heterogeneity of the disorder and the significant impact of the environmental context. As the CSDS model is traditionally run on genetically inbred mice, any contributions in individual differences can be seen by the way of environment or gene x environment interactions. Indeed, researchers have found contributions of

certain candidate genes or pathways of genes involved in mediating the sociability outcome (Bagot et al., 2016; Caradonna et al., 2021; Lorsch et al., 2018). However, the lack of clarity of the genetic contribution to psychiatric disorders makes this association difficult.

Beyond genetics, construct validity can also refer to environmental factors that contribute to the development of disease (Nestler & Hyman, 2010). The CSDS model meets this criterion, as chronic, stressful social events have the ability to induce negative emotional states. In addition, manipulations to the social defeat model, such as differences length, amount of aggression, early or adolescent stress, have all been shown to increase susceptibility and increase the presentations of other depression-like behaviors (Grossman et al., 2022; Lu et al., 2021; Peña, Nestler, & Bagot, 2019). Finally, a key facet is the neural signature of MDD. Through the use of fMRI and post-mortem studies, multiple brain regions have been proposed to contribute an MDD brain-network. In particular, functional connectivity between the subgenual cingulate cortex, amygdala, ventral hippocampus, nucleus accumbens, and VTA have all been proposed to be associated with individual behavioral phenotypes and/or to mediate antidepressant treatment responses in individuals with MDD (Drysdale et al., 2017; Dunlop et al., 2017; Mayberg et al., 2005; Nestler et al., 2002). In the social defeat model, manipulation of these circuits, following stress, have been shown to mediate resilient and susceptible outcomes (Anacker et al., 2016; Bagot et al., 2015, 2016; Chaudhury et al., 2013; Hultman et al., 2018; Kumar et al., 2014; Lobo et al., 2013; Muir et al., 2018, 2020). Therefore, the circuitry that underlies the individual differences in stress reactivity may hold high construct validity in human depression.

In order to assess the predictive validity of the CSDS models, researchers have given a number of antidepressant drugs to mice following defeat to ascertain whether they ameliorate the social avoidance phenotype or modify any other behavioral sequelae associated with vulnerability.

A comprehensive study was conducted that assessed the effects of venlafaxine, fluoxetine, tianeptine in the social interaction test, as well as anxiety-like and depression-like tests (elevated plus maze, novelty suppressed feeding, locomotor activity, forced swim test, and sucrose preference test) (Venzala, García-García, Elizalde, Delagrange, & Tordera, 2012). Repeated treatment with the antidepressant venlafaxine reverted the anhedonia effects seen in sucrose preference, as well as the immobility (learned helplessness) behavior in the forces swim test. Out of the three, only tianeptine was able to reverse the social avoidance phenotype in susceptible mice. In this study, fluoxetine did not show any antidepressant action in defeated mice. In contrast, others have found that fluoxetine is efficacious at preventing the stress-induced social avoidance behavior (Beitia et al., 2005; Berton et al., 2006; Tsankova et al., 2006). This may reflect similar findings to patients with MDD who are classified as responsive or not responsive to certain treatments. These findings underscore the complexity of defining vulnerability in terms of defeat alone or specific to the social avoidance phenotype observed in animals classified as 'susceptible'.

As described above, meeting the requirements for face, construct, and predictive validities in the chronic social defeat stress model is highly dependent on the behavioral output that is being assessed, as well as possibly the researcher's subjective criteria. Due to this, we suggest that the CSDS model should be referred to as representing facets of social stress-related disorders, as a direct comparison to an already heterogeneous disorder in humans adds more complexity than is necessary.

4.4.6 Modeling Specific Aspects of Social Stress-Related Disorders in the Social Defeat Model

Elucidating what other elements, beyond the classification of resilient and susceptible in the social interaction measure, map onto these reactivities is important in increasing our understanding of both adaptive and maladaptive coping strategies in the face of social stressor. For

example, effects of early life stress, increasing the length of defeat, the amount of aggression, using different mouse strains associated with higher anxiety-like behavior have all been shown to increase the proportion of mice who develop the social avoidance phenotype (Dulka et al., 2020; Lu et al., 2021; Oizumi et al., 2019; Peña et al., 2019). Beyond the social interaction measure, traditional downstream behavioral outputs of anxiety-like and depression-like increase in their ability to map on to the vulnerability phenotype, representing multiple factors that are elicited in stress-related disorders (Lu et al., 2021). *Therefore, the CSDS model represents a powerful tool in assessing how manipulation of environmental conditions leads to the individual variation in social stress reactivity, and therefore, the manifestation of certain aspects of stress-related disorders*.

Our findings suggest that coping behaviors used during the initial defeat encounter are predictive of whether resilient or susceptible outcomes arise and that there may be additional contextual manipulations that might also prove useful in shaping coping strategies in individuals at risk for social stress disorders. Understanding the dynamic process in which the initial stress exposure frames future behavior may offer opportunities to interfere and enhance the positive outcomes of this process, while minimizing the cost to the individual. A meta-analysis of psychotherapeutic treatments for those who have SAD indicated that cognitive behavioral therapy (CBT) appears to provide the strongest benefit (Cremers & Roelofs, 2016; Heimberg, 2002; Rodebaugh, Holaway, & Heimberg, 2004). CBT involves several techniques in which the client who experiences any type of anxiety-related or affective disorder works with a therapist to modify existing behaviors through repetition and practice. This results in a cognitive restructuring, providing the individual with behavioral tools to overcome subsequent stressors or fears (Rodebaugh et al., 2004). Individuals who exhibit SAD tend to engage in maladaptive coping strategies, such as avoidance, overestimating the negative consequences of the social encounter,

and apprehension to future social interaction. However, like MDD, SAD is a heterogeneous disorder that can be specific to certain social situations or general social situations and can subsequently affect the type of coping strategy that an individual engages in (Finlayson-Short, Harrison, & Davey, 2021; Hofmann, 2007; Wright, Banerjee, Hoek, Rieffe, & Novin, 2010). This mirrors the individual variation that we found in social coping during the initial defeat encounter in the CSDS model. Using the social defeat model to target coping behaviors used during the initial stress response may provide a fruitful avenue to assess how molecular targets and coping strategies intersect to promote resilience in the face of social stress.

4.5 Remaining Questions and Future Directions

This dissertation has characterized an animal model of individual differences in social reactivity following chronic stress that may provide further insight into the development of vulnerability to social stress disorders. We determined that individual variation in social coping during the initial stress encounter significantly impacts differences in social behavior following stress. This can be further seen at the level of brain-wide neuronal activation patterns that appear to mediate differences in experiencing an agonistic social encounter vs. a positive social encounter, as well as differences in whether social avoidance behavior will be developed following the stress. This dissertation displays a variety of strengths, including an in-depth behavioral characterization of the animal model, the use of novel mouse technology that enabled this entire approach, and identification of circuits that not only represent correlational activation patterns, but also functional connectivity that maps well onto both animal and human studies of emotionality and social behavior. While the work has offered new insights into the neurobehavioral bases of individual differences in social stress reactivity, there still remains a large number of unanswered questions that deserve to be addressed.

We demonstrated significant individual differences in behavioral coping strategy in facilitating and predicting the development of social avoidance behavior following stress. While this initial strategy represents one timepoint of the social defeat experience, how these coping strategies change or are maintained throughout the stress experience is still unknown. It has been shown that a neither a single nor even a small number of social defeat sessions (<5 days) is sufficient to elicit the individual differences in social behavior, but rather the full course (10 days) of repeated defeat is necessary to generate the robust differences in social reactivity (Bagot et al., 2016). We propose that a longitudinal assessment of active and passive coping strategies observed during the entire stress experience will elucidate the stability or dynamic nature that coping strategy has on behavioral outputs. Neural circuitry associated with 'winning' or 'losing' agonistic encounters have shown respective increases or decreases, representing a learning response that feeds-forward to change or maintain coping behaviors in subsequent encounters (Chang & Gean, 2019; Evans et al., 2018; Falkner, Grosenick, Davidson, Deisseroth, & Lin, 2016; Nordman et al., 2020). In addition, environmental manipulations in the social defeat model, such as changes in enrichment status or early life stress, can increase the proportion of socially avoidant mice (Lehmann & Herkenham, 2011; Peña et al., 2019). How coping strategies are or are not changed as a result might provide additional insights into the development of vulnerability.

We demonstrated that selective neural activity patterns that are elicited during the initial defeat encounter may predict the outcome to chronic stress. These activity patterns are highly predictive of the type of coping behaviors that an animal engages in during the initial defeat encounter, especially in the use of escape as a coping strategy. In addition, within susceptible mice, the BMA exhibited the largest number of significant correlations with other brain regions, implicating this hub and its network in conferring vulnerability. Future studies that uncover

whether there is a direct role in these regions in mediating resilient or susceptible outputs, as well as any putative changes in network organization as a result, are necessary.

In addition, the most altered regions that have emerge represent hubs with multiple connections encoded by cellularly diverse subpopulations. Thus, a molecular characterization of the BNST and LSNv regions that are activated by social defeat would represents an important next step in understanding whether Resilient or Susceptible outcomes are a result of distinct molecular activation patterns. Moreover, as this is a transgenic animal, it is possible to carry out a combination of FosTRAP visualization with optogenetic or pharmacological studies. Thus, one can inhibit or activate the BNST and LSNv seed regions during the initial defeat encounter in order to a) 'TRAP' the responses and detect differences in network activation patterns and 2) ascertain whether differences in coping strategies and/or final social outcome change because of these direct manipulations.

An important question is how patterns of neural activation change dynamically across the course of chronic social defeat as it leads to the eventual classification of susceptibility or resilience. How will the brain regions we have identified as nodal changes in terms of activity over time, and how will this impact their associated networks? Will additional brain regions or activation patterns emerge within the same animal as chronic stress continues? Previous work from our lab has shown that Syrian hamsters who are repeatedly exposed to chronic social stress show a complex pattern of change in comparison to acute social stress—several brain areas exhibit habituation of the *c-fos* response to repeated social defeat, but other areas (especially more caudal) exhibit activation levels comparable to what is observed following acute social stress (Kollack-Walker et al., 1999). To assess whether this occurs in the CSDS mouse model, future studies should be conducted using the FosTRAP2 transgenic system to 'TRAP' the initial circuit on Day 1,

continue to place the mice in all 10 days of defeat, conduct the social interaction test to observe final social reactivity, and then expose those same mice to an additional experience of defeat and perfuse for peak Fos protein. The findings from this study will provide for great anatomical resolution in assessing the effects of repeated exposure to stress and any selective differences that result following stress in mediating the final behavioral state.

Together, additional research to address the outstanding questions posed by the studies described in this dissertation will build on important insights into how individual differences in social coping and brain-wide activation patterns during the early stress response influence the development of vulnerability to stress-related disorders. These studies will further develop the role of the chronic social defeat model as a tool in assessing the different factors that contribute to disorders relating to social stress.

4.6 Conclusions

The work in this dissertation underscores the importance of focusing on individual differences in social coping to uncover its underlying neural mechanisms and long-term sequelae in animal models, and to shed light on the neurobiology of mental disorders associated with social stress. Recognizing the complexity of social behaviors and the diversity of behavioral and neural responses to social stimuli may represent a key element in understanding how and why neuropsychiatric disorders appear to be so heterogeneous. How individual differences in behavioral outputs are then reflected in neural circuits that set the tone for future responses and affective states will provide greater progress towards identifying risk factors and treatments for mental health disorders.

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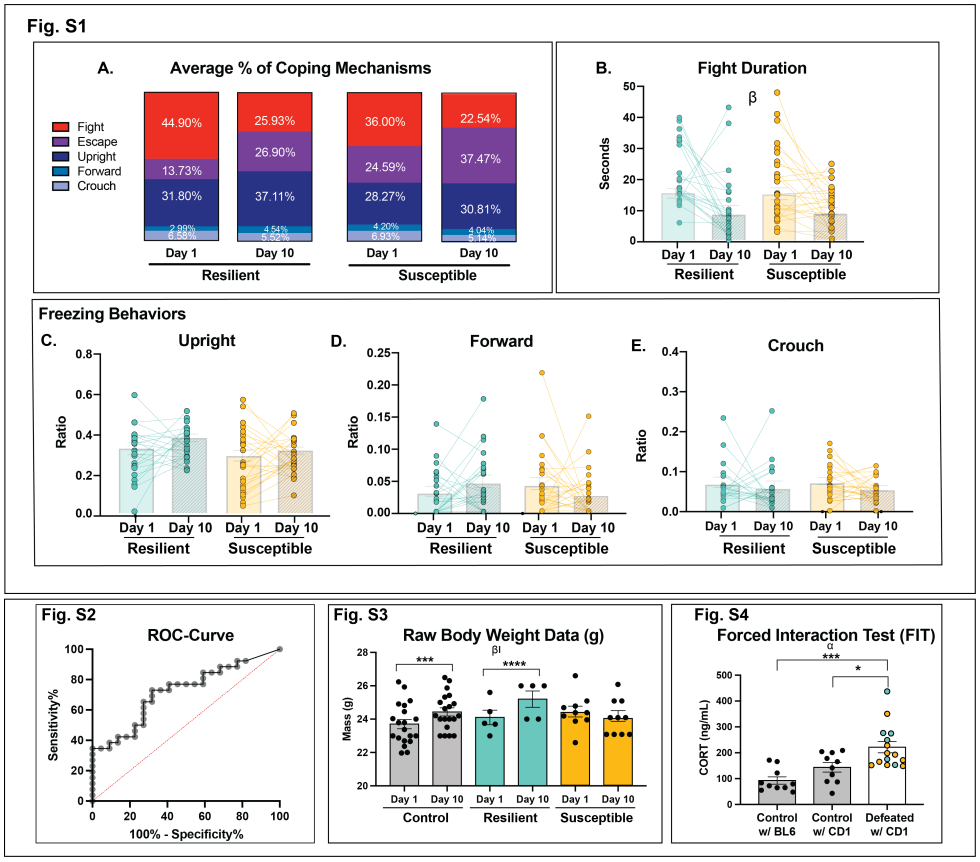
Appendices

Appendix A: Chapter 2 Supplement



Appendix Figure A1: Coping Behaviors Used During Stress

A1A.Parts of a whole bar chart representing the average percent of all coping behaviors used during defeat.

A1B. From Day 1 to Day 10, fight duration decreased similarly between resilient and susceptible groups.

A1C. Ratio of # of Upright Freezes/Total Behaviors Observed was similar between resilient and susceptible mice and did not change due to day.

A1D. Ratio of # of Forward Freezes/Total Behaviors Observed was similar between resilient and susceptible and did not change due to day.

A1E. Ratio of # of Crouch Back Freezes/Total Behaviors Observed was similar between resilient and susceptible and did not change due to day.

A1-2: Receiver Operating Characteristic Curve. Day 1 Escape is highly predictive of resilient and susceptible outcome. Area=0.7203, Std. Error=0.07353, 95% Confidence Interval= 0.5762 to 0.8644, P. Value=0.0091.

A1-3: Raw body weight data from Day 1 and Day 10 between control, resilient, and susceptible mice.

There was a main effect of day and an interaction effect between group and day, as indicated by the significant increase in weight (g) in control and resilient mice, but not susceptible. Importantly, initial body weights were similar across groups (measured at the beginning of defeat).

Figure S4: Forced Interaction Test. Mice were placed in similar conditions to the CR, CSDS, and SI test to look at CORT (ng/mL) expression. There was an overall Group main effect. Post-hoc Tukey tests revealed that CORT (ng/mL) expression of control mice across from BL6 or CD1 did not differ, but there was a significant increase in defeated mice CORT(ng/mL) expression compared to both control conditions.
Significance Codes: Group Main Effect α, Day Main Effect β, Interaction Effect ι, p<0.05, **p< 0.01, ***p< 0.001, p<****0.0001

Table A1: Paired T-test Statistics within Group for Social Interaction Pre-Post Social Interaction

Group Comparison	**T(df)**	**P.Value**
Control Pre-Post	**19(2.653)**	**0.0157**
Defeat Pre-Post	**15(3.855)**	**0.0016**

Table A2: Mann-Whitney T-test Between Groups for Pre-Post Social Interaction

Comparison	**P.Value**	**Mann-Whitney U**
Pre CR – Pre Defeat	P=0.2234	121
Post CR – Post Defeat	**P=0.0035**	**70**

Table A3: Pre-Post Pearson Correlations Table

Behavior	**Comparison**	**Pearson r**	**P.Value**
Pre-Post Social Interaction Test			
	Control Rotation Pre vs. Post	0.1900	0.3988
	Defeat Pre vs. Post	-0.3616	0.1688

Baseline CORT differences

1) We looked at the interactive effects of time of day, pre and post paradigm, and group on plasma corticosterone (CORT) within a multilevel (mixed effects) model (*Equation 1*), with subject ID included as a random effect variable:

   Equation 1: $CORT \sim \beta_0 + \beta_1(Stress) + \beta_2(TimeOfDay) + \beta_3(PreVsPost) + \beta_4(SIScore) + \beta_5(Stress*TimeOfDay) +$, *random=~1|ID*

2) Linear mixed-effects model fit by REML: CORT~ PreVsPostParadigm* TimeOfDay * Group

3) PreVsPostParadigm and TimeOfDay were set as dummy variables

4) Data distributions were examined for normality. SI Ratio was centered. Only CORT data required transformation due to non-normal skewed distribution—the data was log2 transformed and centered.

5) Time of day AM, Pre-Paradigm, and Control mice were set as the reference (intercept)

Table A4: Pre-Post CORT Statistics Table

Predictor	**Estimate**	**Std.Error**	**DF**	**T.Value**	**P.Value**
PreVsPostParadigm	0.2912	0.5028	70	0.5792	0.5643
TimeOfDay	**1.5521**	**0.5028**	**70**	**3.0868**	**0.0029**
GroupResilient	-0.4533	0.7111	33	-0.6376	0.5482
GroupSusceptible	-0.7169	0.6806	33	-0.6126	0.0532
PreVsPostParadigm:TimeOfDay	-0.4102	0.7055	70	-0.5815	0.5628
PreVsPostParadigm:GroupResilient	0.1104	1.0056	70	0.1098	0.9128
PreVsPostParadigm:GroupSusceptible	0.8971	0.8369	70	1.0718	0.2875
PreVsPostParadigm:TimeOfDay:GroupResilient	-0.3730	0.9737	70	-0.3830	0.7028
PreVsPostParadigm:TimeOfDay:GroupSusceptible	1.1639	0.8210	70	1.41760	0.1607

Table A5: Coping Behaviors During Stress 2x2 Mixed ANOVA Table

Behavior	Effect	F(df)	P.Value	Partial Eta Squared
Fight Duration				
	Group	0.03(1,44)	0.860	<0.001
	Cohort	1.34(2,44)	0.272	0.027
	Day	16.20(1,44)	**<0.001**	0.166
	Group:Day	0.19(1,44)	0.665	0.002
	Cohort:Day	0(2,44)	>0.999	<0.001
Fight				
	Group	3.45(1,44)	0.070	0.054
	Cohort	0.13(2,44)	0.876	0.004
	Day	39.07(1,44)	**<0.001**	0.266
	Group:Day	0(1,44)	0.956	<0.001
	Cohort:Day	3.05(2,44)	0.057	0.054
Escape				
	Group	7.07(1,44)	**0.011**	0.092
	Cohort	1.34(1,44)	0.193	0.047
	Day	29.92(2,44)	**<0.001**	0.199
	Group:Day	1.09(1,44)	0.303	0.009
	Cohort:Day	6.07(2,44)	0.054	0.072
Upright				
	Group	1.78(1,44)	0.189	0.020
	Cohort	1.79(2,44)	0.178	0.039
	Day	1.94(1,44)	0.170	0.022
	Group:Day	0.95(1,44)	0.335	0.011
	Cohort:Day	1.49(2,44)	0.237	0.033
Forward*				
	Group	0.34(1,44)	0.561	0.004
	Cohort	4.32(2,44)	**0.019**	0.081
	Day	0.90(1,44)	0.349	0.001
	Group:Day	0.01(1,44)	0.917	<0.001
	Cohort:Day	3.49(2,44)	**0.039**	0.080
Crouch*				
	Group	0.46(1,44)	0.502	0.003
	Cohort	4.44(2,44)	**0.018**	0.064
	Day	2.44(1,44)	0.125	0.035
	Group:Day	0.33(1,44)	0.571	0.005
	Cohort:Day	2.18(2,44)	0.125	0.062

*Forward and Crouch behaviors on average were utilized <5% and <7% of the time overall, therefore the variability as shown by the cohort effect is most likely due to the extremely low occurrences

-No Interaction effects were found, thus post-hoc tests were not conducted

Table A6: One-Way ANOVA Table

Behavior	F(df)	P.Value	Effect Size
Open Field			
% Time Spent in Center (Group Effect)	6.689(2,31)	**0.0039**	0.51
Total Distance Traveled (Group Effect)	1.718(2,31)	0.1965	0.23
Forced Swim Test			
Time Spent Immobile (sec) (Group Effect)	3.93(2,31)	**0.0298**	0.38
Von Frey			
Force of filament used (g) (Group Effect)	5.163(2,33)	**0.0112**	0.44
Forced Interaction			
CORT (ng/mL) with CD1 Only (Group Effect)	4.56(2,23)	**0.0214**	0.47
CORT (ng/mL) All Conditions (Group Effect)	11.44(2,32)	**0.0002**	0.62
Body Weight			
Change in Body Weight Day 10 - Day (Group Effect)	18.6(2,33)	**<0.0001**	0.71

Table A7: Linear Model for FIT CORT differences in Stressed Mice

Effect	Estimate	Std.Error	T.Value	P.Value
SIRatio	**125.36**	**45.33**	**2.765**	**0.01711**
BodyWeightChange	-31.62	24.05	-1.319	0.21170

Table A8 Post-Hoc Tukey Test Table

Behavior	Group Comparison	P.adj
Open Field % Time Spent in Center		
	Control-Resilient	**0.0074**
	Control-Susceptible	**0.0451**
	Resilient-Susceptible	0.8648
Forced Swim Test- Time Spent Immobile		
	Control-Resilient	0.1847
	Control-Susceptible	0.2712
	Resilient-Susceptible	**0.0231**
Von Frey Test		
	Control-Resilient	0.8496
	Control-Susceptible	**0.0087**
	Susceptible-Resilient	0.0691
Forced Interaction Test (CD1 CORT)		
	Control - Resilient	**0.0162**
	Control - Susceptible	0.4616
	Susceptible - Resilient	0.1413
Forced Interaction Test (overall CORT)		
	Control C57BL6J - Control CD1	0.2301
	Control C57BL6J - Defeat CD1	**0.0211**
	Control CD1 - Defeat CD1	**0.0002**
Body Weight Change Day 10-Day 1		
	Control-Resilient	0.4574
	Control-Susceptible	**<0.0001**
	Resilient-Susceptible	**0.0003**

Table A9: ANOVA Table Fig S3

Behavior	Effect	F(df)	P.Value	Partial eta squared
Raw Body Weight Data				
	Group	0.6(2,32)	0.553	0.033
	Day	14.52(1,32)	**<0.001**	0.037
	Group:Day	12.31(2,32)	**<0.001**	0.062

Table A10: Pearson Correlations Table for Figures 2.4 and 2.5

Behavior	Comparison	Pearson r	P.Value
Open Field			
	Control SI Ratio vs. % Time Spent in Center	0.381	0.1701
	Defeat SI Ratio vs. % Time Spent in Center	-0.172	0.5393
Forced Swim Test			
	Control SI Ratio vs. Time Spent Immobile (sec)	0.417	0.0757
	Defeat SI Ratio vs. Time Spent Immobile (sec)	-0.264	0.3413
Von Frey Test			
	Control Force (g) vs. SI Ratio	-0.132	0.6139
	Defeat Force (g) vs. SI Ratio	**0.581**	**0.0144**
Forced Interaction Test			
	Control CORT ng/ml vs. SI Ratio (CD1)	0.191	0.5963
	Control CORT ng/ml vs. SI Ratio (C57BL6J)	-0.036	0.9195
	Defeat CORT ng/ml vs. SI Ratio	0.535	**0.0260**
Body Weight Changes			
	Control SI Ratio vs. Body Weight (g)	0.693	0.0943
	Defeat SI Ratio vs. Body Weight (g)	0.702	**0.0035**

Table A11: 2x2 Mixed ANOVA Table for Fig. 2.6

Behavior	Effect	F(df)	P.Value	Partial eta squared
Novel C57BL6J Social Interaction				
	Group	13.70(2,23)	**<0.001**	0.443
	Strain	2.360(1,23)	0.1380	0.033
	Group:Strain	18.11(2,23)	**<0.001**	0.344
Black Swiss Social Interaction				
	Group	13.270(2,20)	**<0.001**	.350
	Strain	0.790(1,20)	0.3860	0.023
	Group:Strain	11.240(2,20)	**<0.001**	0.400

Table A12: Figure 2.6 One-Way ANOVA Table

Behavior	**F(df)**	**P.Value**	**Effect Size**
Strain Specificity: C57BL6/J			
Social Group (Group Effect)	0.373(2,23)	0.6930	0.17
Strain Specificity: CD1 Condition of C57BL6/J			
Social Group (Group Effect)	34.080(2,23)	**<0.0001**	0.85
Strain Specificity: Black Swiss			
Social Group (Group Effect)	0.120(2,20)	0.8880	0.11
Strain Specificity: CD1 Condition of Black Swiss			
Social Group (Group Effect)	19.390(2,20)	**<0.0001**	0.79

Table A13: Figure 2.6 Post-Hoc Tukey Tests

Behavior	**Group Comparison**	**P.adj**
Strain Specificity: C56BL6/J Interaction		
	Control - Resilient	0.8666
	Control - Susceptible	0.9294
	Resilient - Susceptible	0.6764
Strain Specificity: CD1 Condition of C56BL6/J Interaction		
	Control - Resilient	0.4912
	Control - Susceptible	**<0.0001**
	Resilient - Susceptible	**<0.0001**
Strain Specificity: Black Swiss		
	Control - Resilient	0.9153
	Control - Susceptible	0.8998
	Resilient - Susceptible	0.9956
Strain Specificity: CD1 Condition of Black Swiss		
	Control - Resilient	**0.0346**
	Control - Susceptible	**0.0000**
	Resilient - Susceptible	**0.0049**

Appendix B: Chapter 3 Supplement

Table B1: Brain Regions Selected for Exploratory Approach (73)

Acronym	Name
ACC	Anterior cingulate area
PL	Prelimbic area
IL	Infralimbic area
OA	Orbital area
Pir	Piriform area
Co	Cortical amygdalar area
aPir	Piriform-amygdalar area
CA1	Field CA1
CA2	Field CA2
CA3	Field CA3
DG	Dentate gyrus
EPN	Endopiriform nucleus
LA	Lateral amygdalar nucleus
BLA	Basolateral amygdalar nucleus
BMA	Basomedial amygdalar nucleus
PA	Posterior amygdalar nucleus
CPu	Caudoputamen
NAc	Nucleus accumbens
OT	Olfactory tubercle
LSNc	Lateral septal nucleus/ caudal (caudodorsal) part
LSNrv	Lateral septal nucleus/ rostral (rostroventral) part
LSNv	Lateral septal nucleus/ ventral part
SF	Septofimbrial nucleus
AAA	Anterior amygdalar area
BNAOt	Bed nucleus of the accessory olfactory tract
CeA	Central amygdalar nucleus
IAN	Intercalated amygdalar nucleus
MeA	Medial amygdalar nucleus
GPe	Globus pallidus/ external segment
GPi	Globus pallidus/ internal segment
SI	Substantia innominata
MRn	Magnocellular nucleus
MSc	Medial septal complex
MSn	Medial septal nucleus
DBB	Diagonal band nucleus
TRS	Triangular nucleus of septum
BNST	Bed nuclei of the stria terminalis
BNAC	Bed nucleus of the anterior commissure
LPN	Lateral posterior nucleus of the thalamus

PC	Posterior complex of the thalamus
POL	Posterior limiting nucleus of the thalamus
SGn	Suprageniculate nucleus
EN	Ethmoid nucleus of the thalamus
AvN	Anteroventral nucleus of thalamus
AmN	Anteromedial nucleus
AdN	Anterodorsal nucleus
IAM	Interanteromedial nucleus of the thalamus
IAD	Interanterodorsal nucleus of the thalamus
LD	Lateral dorsal nucleus of thalamus
IMT	Intermediodorsal nucleus of the thalamus
MD	Mediodorsal nucleus of thalamus
SMT	Submedial nucleus of the thalamus
PR	Perireunensis nucleus
PVT	Paraventricular nucleus of the thalamus
NR	Nucleus of reuniens
RN	Rhomboid nucleus
CM	Central medial nucleus of the thalamus
OPC	Paracentral nucleus
CL	Central lateral nucleus of the thalamus
PF	Parafascicular nucleus
PIT	Posterior intralaminar thalamic nucleus
GpVT	Geniculate group/ ventral thalamus
Mhb	Medial habenula
Lhb	Lateral habenula
PVZ	Periventricular zone
PVNr	Periventricular region
MHZ	Hypothalamic medial zone
LHZ	Hypothalamic lateral zone
VMH	Ventromedial hypothalamic nucleus
AH	Anterior hypothalamic nucleus
ME	Median eminence
VTA	Ventral tegmental area
PAG	Periaqueductal gray

Table B2: Brain Regions Selected for Candidate Approach (26)

Acronym	**Brain Region**
PL	Prelimbic area
IL	Infralimbic area
CA1	Field CA1
CA2	Field CA2
CA3	Field CA3
DG	Dentate gyrus
LA	Lateral amygdalar nucleus
BMA	Basomedial amygdalar nucleus

Cpu	Caudoputamen
LSNc	Lateral septal nucleus/ caudal (caudodorsal) part
LSNr	Lateral septal nucleus/ rostral (rostroventral) part
LSNv	Lateral septal nucleus/ ventral part
CeA	Central amygdalar nucleus
IAN	Intercalated amygdalar nucleus
MeA	Medial amygdalar nucleus
BNST	Bed nuclei of the stria terminalis
PVT	Paraventricular nucleus of the thalamus
MHb	Medial habenula
LHb	Lateral habenula
PVH	Periventricular Region
VMH	Ventromedial hypothalamic nucleus
NAc	Nucleus accumbens
VTA	Ventral tegmental area
LHZ	Hypothalamic lateral zone
GVT	Geniculate group/ ventral thalamus
BLA	Basolateral amygdalar nucleus