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Multidimensional Predictors of Susceptibility and Resilience to Social Defeat Stress

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Abstract

Background: Previous studies identified several separate risk factors for stress-induced disorders. However, an integrative model of susceptibility versus resilience to stress including measures from brain-body domains is likely to yield a range of multiple phenotypic information to promote successful adaptation to stress.

Methods: We used computational and molecular approaches to test whether (i) integrative brain-body behavioral, immunological and structural domains characterized and predicted susceptibility

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or resilience to social defeat stress (SDS) in mice, and (ii) administration of acetyl-L-carnitine (LAC) promoted resilience at the SDS paradigm.

Results: Our findings identified multidimensional brain-body predictors of susceptibility versus resilience to SDS. The co-presence of anxiety, decreased hippocampal volume and elevated systemic interleukin-6 characterized a susceptible phenotype that developed behavioral and neurobiological deficits after exposure to SDS. The susceptible phenotype showed social withdrawal and impaired transcriptomic-wide changes in ventral dentate gyrus after SDS. At the individual level, a *computational approach* predicted if a given animal developed SDS-induced social withdrawal, or remained resilient, based on the integrative *in-vivo* measures of anxiety and immune system function. Finally, we provide initial evidence that administration of LAC promoted behavioral resilience at the SDS paradigm.

Conclusions: The current findings of multidimensional brain-body predictors of susceptibility versus resilience to stress provide a starting point for *in-vivo* models of mechanisms predisposing apparently healthy individuals to develop the neurobiological and behavioral deficits resulting from stress exposure. This framework can lead to novel therapeutic strategies to promote resilience in susceptible phenotypes.

Keywords

Risk factors; Acetylcarnitine; Epigenetic; Individual Differences; Biomarkers; Phenotype

Introduction

Why do some individuals show neurobiological and behavioral deficits after exposure to stress(1, 2), whereas others maintain adaptive capacity and show resilience (3–5)? Although vast literature characterized the susceptible and resilient phenotypes after exposure to stress (6–9), less is known about the mechanisms predisposing apparently healthy individuals to develop maladaptive coping strategies from those that confer resilience.

Prediction of individuals at risk of developing stress-induced disorders has been based largely upon single risk factors. For example, previous work identified increased anxiety-like behavior induced by a glucocorticoid overactivation as a risk factor for development of stress-induced glutamatergic dysfunction in the ventral hippocampus (vHIPP) with corresponding depressive-like traits in susceptible mice (5, 8, 10). Variability in affective regulation has also been associated with structural differences in limbic brain areas, such as the medial prefrontal cortex (9), that have dense bidirectional connectivity with the vHIPP (1, 3, 11). Further supporting a role for affective dysregulation as a risk factor for susceptibility to stress, previous work showed increased anxiety-like behavior associated with social hierarchy in rodents that develop stress-induced depressive-like traits (12). In addition to behavioral risk factors, dysregulation of the immune system as manifested by heightened interleukin-6 (IL-6) release has been linked to susceptibility to social defeat stress (SDS) (7, 13). Although multiple single risk factors of susceptibility to stress have been discovered (7, 8, 14, 15), there is a need to determine integrative measures of multiple brain-body factors that most likely can be more accurate than a single risk factor to explain the complexity of individual responses to stress.

An integrative model of susceptibility versus resilience to stress integrating brain-body measures is likely to yield a range of multiple phenotypic information to promote successful adaptation to stress (16, 17). Groundbreaking findings showed pro-resilient actions of the glutamatergic agent ketamine at the SDS paradigm (18, 19). Furthermore, a growing literature from our group and others suggested acetyl-L-carnitine (LAC) as a novel rapid-acting glutamatergic agent to ameliorate stress-induced neurobiological and behavioral impairments (10, 20–28). Administration of LAC, a drug with a good profile of tolerability, leads to rapid behavioral actions by acetylating histones to regulate the expression of the metabotropic glutamate receptor type-2 (mGlu2) and corresponding structural plasticity. The mGlu2 receptor is a key inhibitor of spontaneous glutamate release. However, potential pro-resilient actions of LAC remain to be elucidated.

Here, we aimed at determining integrative measures of susceptibility versus resilience to stress and test whether administration of LAC can serve to promote successful adaptation to stress. Specifically, by using computational, behavioral and molecular approaches, first we tested whether a combination of brain-body factors characterized and predicted susceptible or resilient phenotypes at the SDS. Second, we tested whether administration of LAC exerts pro-resilient action at the SDS paradigm.

Methods

More information is available in the supplementary information (SI).

Behavioral assessment prior or after SDS

Light Dark Test (LDT) as screening method for individual susceptibility was performed as previous described(8), and detailed in SI.

Elevated Plus Maze (EPM): one week after the LDT screening, mice were tested at the EPM as previously described (29). More details in SI.

Social interaction test was performed at the end of the SDS paradigm as previously described(30). More details in SI.

Immunological assessment

Flow cytometry—Flow cytometry studies were performed using a LSRII Fortessa (Becton Dickinson) and analyzed using FlowJo software (Tree Star). Fluorochrome or biotin-conjugated mAbs specific for mouse CD11b (clone M1/70), CSF-1R (also called CD115) (clone AFS98), Ly6C (clone HK1.4), Ly6G (clone 1A8) and the secondary reagents (allophycocyanin, peridinine chlorophyll protein, and phycoerythrin-indotricarbocyanine-conjugated streptavidin) were obtained from BD Biosciences, eBioscience, or Biolegend30.

Leukocyte Isolations/Immune Challenge.

Whole blood (200μL) was transferred to a 15-mL conical tube and mixed with 2 mL of complete media (RPMI 1640, 20% horse serum,10% FBS, 2 mM l-glutamine, 100 units per mL of penicillin, and 100 μg/mL streptomycin). The blood/media mixture was layered over an equal volume of Ficoll-Paque Plus (GE Healthcare). Samples were centrifuged (790 χ g,

15 min, 25 °0) to form a buffy coat layer. Cells were removed, washed in BEP solution (PBS with 0.5% BSA and 2 mM ETDA), and centrifuged (529 × g, 8 min, 25 ‘C). The supernatant was removed, and cells were re-suspended in 200 μL of BEP solution. Cell aliquots were stained with trypan blue, and cells were counted on a hemocytometer. Cells were plated at 1 × 10^6 cells per well in 1 mL of media or media + 34 μg/mL lipopolysaccharide (LPS, Sigma) and stored for 24h at 37C with 5% CO_2. After 24 h, cells and media were removed from plates, centrifuged (2,348 × g, 5min), and supernatant was removed and stored at −80 until IL-6 analysis.

IL-6 Enzyme linked immunosorbent assays (ELISA) was performed as previously described(7).

Brain imaging and ex-vivo magnetic resonance imaging (MRI) scans

One week after LDT testing, brains were processed for brain imaging as previously described(31).

Stress paradigm

Social defeat stress (SDS) was performed as previously described(30).

Pharmacological approach

Acetyl-L-carnitine (LAC)—LAC (Sigma-Aldrich, St Louis, MO, USA) was dissolved in the drinking water and administered for 3 full days prior to the end of the SDS paradigm at a concentration of 0.3%. A vehicle solution of water only was used as control. Mice were kept with either vehicle or LAC solution treatment until behavioral testing. Reports from previous studies(10, 21) were used to assure that the animals’ fluid intake and hydration state were not altered by the oral LAC administration. This was achieved by evaluating skin turgor, body weight and daily food and fluid consumption for 3 days.

Gene expression and Bioinformatics

Tissue processing for RNAseq gene expression and bioinformatics analysis was performed as previously described(10, 32). Significance was set at uncorrected $p<0.05$ for broad pattern identification and fold-change (FC) threshold was set at ±30%. Significance was set at FDR<0.05 for pathway analysis by using the Reactome Pathways in Panther(33, 34). More details in SI.

Computational approach to classify susceptible and resilient phenotypes

We used a computational approach in R to predict if a given animal developed SDS-induced social withdrawal, or remained resilient based on the LDT and IL-6 scores. Based on previous literature, thresholds of 115 seconds and 100pg/mL for the LD and IL-6 scores, respectively(7, 8) were used to classify mice predicted to become susceptible (i.e.: LD score < 115 and IL-6 score > 100) or remain resilient (i.e.: LD score > 115 and IL-6 score < 100). Mice with LD score < 115 and IL-6 score < 100 or LD score > 115 and IL-6 score > 100 were classified as uncertain prediction by the R algorithm. We also compared the numbers of predicted susceptible and predicted resilient with the number of mice that indeed became

susceptible (actual susceptible) or remained resilient (actual resilient) based on the social interaction outcome after SDS.

Statistics

Statistical analyses were performed using one- or two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, or the two-tailed unpaired Student's t tests as appropriate. The number of mice per group used in each experiment is reported in the corresponding figure legends. Likewise, significance and F-test values are reported in the caption of each figure.

Results

Interrelated predictors characterize the HS and LS bio-behavioral phenotypes

We recently introduced a modified version of the light dark test (LDT) as a rapid screening tool to identify animals susceptible to stress within an inbred population of mice (8). Mice that displayed increased anxiety-like behavior showed elevated expression of hippocampal mineralocorticoid receptors (MR) before any applied stress. This subset of mice showed a decrease in expression of mGlu2 receptors in hippocampus with corresponding depressive-like behavior after exposure to chronic restraint stress (8, 21, 35). Here, we used the LDT (Fig.1A) to first test whether anxiety-like behavior is associated with aberrant activation of the immune system and hippocampal volumetric changes. We chose the LDT method because of the simplicity of the test that allows to minimize any unwanted stress effects.

We found that mice designated as high susceptible (HS) using the LDT, and characterized by decreased time spent in the light chamber (Fig.1B), also spent less time in the center of the open chamber as compared to mice designated as low susceptible (LS) (Fig.1C). Further supporting the occurrence of anxiety-like behavior in the HS phenotype, we found that mice designated as HS by the LDT spent less time and showed a decreased number of entries in the open arms of the elevated plus maze (EPM) as compared to LS mice (SI Fig. 1A–B). Next, we investigated whether the HS and LS phenotypes were associated with volumetric changes in the hippocampus, a brain region implicated in anxiety-like behavior (8, 36). Exploratory analyses showed a positive correlation between the volume of the hippocampus and degree of time spent in the light chamber of the light-dark box. Mice that displayed the highest anxiety-like behavior had the smallest hippocampal volume prior to any applied stressor (Fig.1D–E).

To further characterize the HS and LS phenotypes, we examined several markers of the systemic immune system, including the pro-inflammatory cytokine IL-6, a known marker of susceptibility to SDS (7). Mice identified through the LDT as HS had higher circulating neutrophil counts and a trend towards higher counts of inflammatory monocytes prior to any stress exposure (SI Fig. 2A–B). Furthermore, there was a negative correlation between LPS-stimulated IL-6 release and LDT scores in that animals with the highest LPS-stimulated levels of IL-6 spent the shortest time in the light chamber of the LDT (Fig. 1F). These data suggest a relationship between the anxiety-like phenotype of HS mice and an exacerbated basal immune response. No basal differences in the CD45 leukocytes population or IL-6

without LPS stimulation were detected (SI Fig.2C–D). Together these data show distinct bio-behavioral phenotypes of the HS and LS mice.

The HS and LS phenotypes modulate susceptibility and resilience to SDS

First, we tested whether the HS and LS bio-behavioral phenotypes could modulate the behavioral responses to stress. To test this hypothesis, we subjected both HS and LS mice to 10 days of SDS (Fig.2A), and evaluated social interaction behavior at the end of the SDS paradigm. We found that HS phenotype, but not the LS phenotype, showed social withdrawal 24 hours after the last defeat episode as compared with unstressed control mice (Fig.2B). These data show that the HS and LS bio-behavioral phenotypes differed in their behavioral responses to stress with development of SDS-induced social withdrawal in HS mice, while LS mice as a group remained resilient to SDS.

Next, we used RNA sequencing (RNAseq) to capture transcriptome-wide alterations in HS and LS mice after exposure to SDS (HS-SDS and LS-SDS) as compared to the unstressed control group (Ctrl). We narrowed our sequencing approach to the ventral dentate gyrus (vDG, Fig.2C), a brain area recently implicated in resilience to stress (10). The HS-SDS mice showed a distinct gene expression profile as opposed to the LS-SDS mice. Indeed, SDS altered expression of 372 genes (fold change>1.3, p value<0.05) in HS mice compared to the 612 genes in LS mice with 124 overlapping gene changes between HS-SDS and LS-SDS mice (Fig.2D–E). The higher number of differentially expressed genes in the transcriptomic profile of mice resilient to SDS than in that of mice that were susceptible is in agreement with the notion of resilience to stress as being an active process, and not simply the lack of susceptibility (3–6).

Notably, enrichment pathway analyses revealed a unique profile of pathways differentially regulated by SDS in HS and LS mice. Specifically, these data showed the involvement of pathways related to acyltransferase activity and fatty acids, known metabolic targets regulated by LAC, in susceptibility versus resilience to SDS. Further supporting the involvement of metabolic pathways in the responses to SDS, *ApoC3* was among the top ten genes that were selectively altered in HS-SDS mice but not in LS-SDS mice (SI Table 1). *ApoC3* is a gene involved in the maintenance of homeostasis of triglycerides.

Therefore, our data show that the HS phenotype, which is characterized by co-presence of anxiety, elevated leukocyte-derived IL-6 and small hippocampal volume before any applied stressor became later susceptible to SDS, manifesting the neurobiological and behavioral stress-induced deficits.

Multidimensional predictors of the behavioral responses to stress

Given the findings above showing that the HS and LS phenotypes modulate the responses to stress, we used a computational approach to test whether the LDT and IL-6 scores could predict if a given animal will develop SDS-induced social withdrawal, or remained resilient. We reasoned that a classifier that integrates a-priori multidimensional and yet distinct markers of anxiety-like behavior and immune system would predict susceptible phenotypes at the SDS paradigm better than the individual measures. Based on previous literature (7, 8), we used thresholds of 115 seconds and 100pg/mL for the LD and IL-6 scores, respectively

to classify mice predicted to become susceptible (i.e.: LD score < 115 and IL-6 score > 100) or remain resilient (i.e.: LD score > 115 and IL-6 score < 100) (Fig.3A). Next, we compared the numbers of predicted susceptible and predicted resilient with the number of mice that indeed became susceptible (actual susceptible) or remained resilient (actual resilient) based on the social interaction outcome after SDS. A confusion matrix in Fig. 3B depicts the predicted and actual numbers of susceptible and resilient mice. The classifier predicted 18 mice to develop social withdrawal after SDS, and 10 to show behavioral resilience. After SDS, 16 out of the 18 predicted susceptible mice manifested social withdrawal while 2 did not (Fig. 3B). With regard to the prediction of resilience to SDS, 6 out of the 10 predicted resilient mice were actual resilient while 4 developed social withdrawal (Fig. 3B). To quantify the estimated probabilities that the predicted phenotypes reflected the actual numbers of mice exhibiting or not social withdrawal after SDS, we calculated the sensitivity and specificity. The classifier predicted susceptibility and resilience to SDS with a sensitivity of 80% (i.e.: prediction of susceptibility) and specificity of 75% (i.e.: prediction of resilience) (Fig.3B). Next, we tested the strength of the classifier versus the categorization of the individual measures by using the same thresholds and algorithm as above. We found that the combined measures predict susceptibility with a stronger power than either individual measure as showed by a higher sensitivity of 80% as compared to 76% for the LD categorization alone (SI Fig.3A) and 72% for the IL-6 categorization alone (SI Fig.3B). We reason that either individual measures miss-categorize some mice that instead are classified as ‘uncertain’ prediction by the combined measures (gray dots in Fig.3A). These findings suggest that combining multidimensional a-priori biomarkers has a high ability to predict the behavioral deficits resulting from exposure to SDS as schematized in Figure 3C.

Rapid Pro-resilient Effects of Acetyl-L-carnitine (LAC) at the SDS paradigm

Previous research suggested LAC as a novel rapid acting antidepressant candidate. However it remains to be fully explored whether LAC can serve to promote resilience to stress. Given the findings above from the RNAseq analyses showed effects of SDS in regulating known pathways involved in the biology of LAC signaling (e.g.: fatty acids and acyltransferase pathways), we tested whether administration of LAC 3 days before the end of the SDS (Fig. 4B) led to pro-resilient effects at the SDS paradigm. First, we found that while SDS decreased locomotor activity regardless of treatment, SDS mice that received LAC or vehicle showed no difference in the distance travelled in the SI test (SI Fig.4). These data showed that administration of LAC does not affect locomotor activity. Next, we found that administration of LAC normalized social interaction in stressed mice to the degree that ratios were similar to the level of unstressed controls (Fig.4A–B), and were significantly different from stressed mice that received vehicle (Fig.4A–B). Likewise, SDS mice that received administration of LAC showed a decrease in social avoidance ratio to the levels of unstressed control mice (Fig.4C). These data show that administration of LAC opposed the behavioral effects of SDS. These results indicate rapid actions of LAC to enhance behavioral resilience to SDS.

Discussion

We report that multidimensional biomarkers spanning behavioral, systemic and brain domains characterize susceptible and resilient phenotypes, and predict the individual neurobiological and behavioral responses to stress. To the best of our knowledge, this study also provides the first evidence of rapid pro-resilient effects of the epigenetic modulator of glutamatergic function acetyl-L-carnitine (LAC) at the SDS paradigm. Our multidimensional predictive model can lead to a novel framework that can be applied to study mechanisms predisposing apparently healthy animals (susceptible phenotypes) to develop neurobiological and behavioral impairments resulting from exposure to stress from those that confer resilience. The same computational algorithm could be applied to translational research to study mechanisms of development of psychiatric disorders.

A-priori multidimensional constructs define phenotypes of susceptibility versus resilience to stress. We found an association between increased anxiety-like behavior and dysregulation of the immune system along with decreased hippocampal volume prior to stress in apparently healthy mice that developed SDS-induced impairments. Supporting the notion that susceptibility to stress is linked to dysregulation of both brain and systemic functions (3, 5, 37), our current findings provide an integrative model including multiple brain-body phenotypic information that modulate an individual predisposition to the responses to stress. Together with previous findings of glucocorticoid overactivation in the HS phenotype, we propose a model in which HPA axis hyperactivity and heightened inflammation in apparently healthy HS mice may lead to compensatory changes in hippocampal volume that predispose to a lack of flexible adaptation to stress exposure. Our findings also support future studies aimed to investigate whether potential pre-existing differences in neuron morphology (e.g.: structural plasticity) may explain the differential hippocampal volumes as a function of the LDT score in the HS and LS phenotypes.

In agreement with the concept of precision medicine, these findings also suggest that a richer set of bio-behavioral factors is likely to yield a more accurate prediction of the individual responses to stress. By using the identified biomarkers, our computational classifier successfully predicted the behavioral responses of a given animal to SDS with a power stronger than that of individual measures. Indeed, the classifier predicted susceptibility and resilience to SDS with a probability of 80% and 75%, respectively. The high predictive ability of the classifier is of particular importance because it can provide an integrative framework for future research to study mechanisms predisposing (or protecting from) apparently healthy individuals to develop the deficits resulting from exposure to chronic stress. This same computational approach integrating multiple phenotypic information can also be applied in humans to possibly predict development of depressive disorders.

Greater understanding of the role of the glutamatergic agent LAC in modulating the effects of stress on brain plasticity may lead to precision medicine interventions to mitigate susceptibility to stress, and ultimately, vulnerability to depressive disorders. In agreement with the previously documented rapid antidepressant-like action of LAC, the pro-resilient responses to administration of LAC were seen after just a few days of administration at the

SDS paradigm (10, 20, 22, 28). Previous research also showed that a deficiency in the endogenous levels of LAC is a signature of hippocampal glutamatergic dysfunction (10, 20–27). Supplementation with LAC has been associated with improvement of glutamate homeostasis through elevation of a stress-induced decrease in expression of mGlu2 receptors in the vDG.

Reinforcing the importance of brain-body communication, LAC also ameliorates IR(22), a metabolic dysfunction associated with inflammation (17). Inflammatory/metabolic abnormalities have also been observed in mice susceptible to SDS as manifested by increased body weight and insulin insensitivity 4 weeks after discontinuing the SDS paradigm (25). Furthermore, LAC is known to have beneficial effects to improve efficiency of mitochondrial function and reduce free radical formation and, therefore, inflammatory tone(38). Thus it is expected that other mechanisms that result from lack of central and systemic resilience (17, 39) may be implicated in the mechanism of action of LAC. One attractive hypothesis for future research on determining possible mechanistic targets in the pro-resilient action of LAC is that administration of LAC exerts pro-inflammatory effects by decreasing IL-6 levels in susceptible phenotype to promote successful adaptation to stress.

Future studies are also needed to further characterize the here identified HS and LS phenotypes as well as to investigate epigenetic/environmental factors early in life that determine the origin of the distinct bio-behavioral phenotypes. Given the observed clustering of risk factors is found within an inbred, genetically similar strain, a genetic liability alone is likely not driving these divergent phenotypes (40, 41). Early life stress and variations in maternal care of offspring are critical factors underlying the development of individual differences in responses to stress through epigenetic mechanisms (42). Recent studies showed that early life stress encodes lifelong susceptibility to social defeat stress (32). Early life stress, such as childhood emotional trauma, is also a determinant of a deficiency of the epigenetic modulator of glutamatergic function LAC in patients suffering from MDD (43). Recently, we reported decreased LAC levels in two independent populations of patients suffering from major depression (27, 43). The LAC deficiency was greater in individuals with severe, early-onset and treatment-resistant depression that was also associated with high rates of childhood emotional trauma (27, 43). Exposure to childhood trauma has also been linked to inflammatory states, such as insulin resistance (IR) (44), a metabolic dysfunction associated with both a LAC deficiency, and decreased hippocampal volume (45). This knowledge will also inform treatment decision. If a LAC deficiency and the associated consequences are the result of early life adversity, one could consider the possibility that use of LAC could have wide-ranging effects in mitigating the effects of early life adversity on individual susceptibility to stress, and ultimately, vulnerability to depressive disorders that are also accompanied by systemic disorders involving inflammatory processes (46).

In conclusion, the current findings of a bio-behavioral phenotype of susceptibility to stress prompt further basic and translational research to study the mechanisms that lead apparently healthy individuals to manifest the neurobiological, systemic and behavioral effects of stress. The multidimensional computational approach to predict animals at risk is a model that can be applied to human studies of depression vulnerability, and more generally disease

development and associated consequences. Our findings also compel further basic and translational research on the pro-resilient effects of LAC as such a treatment may promote resilience in a way different from traditional pharmacological agents that require prolonged prophylactic treatment to prevent recurrent depressive episodes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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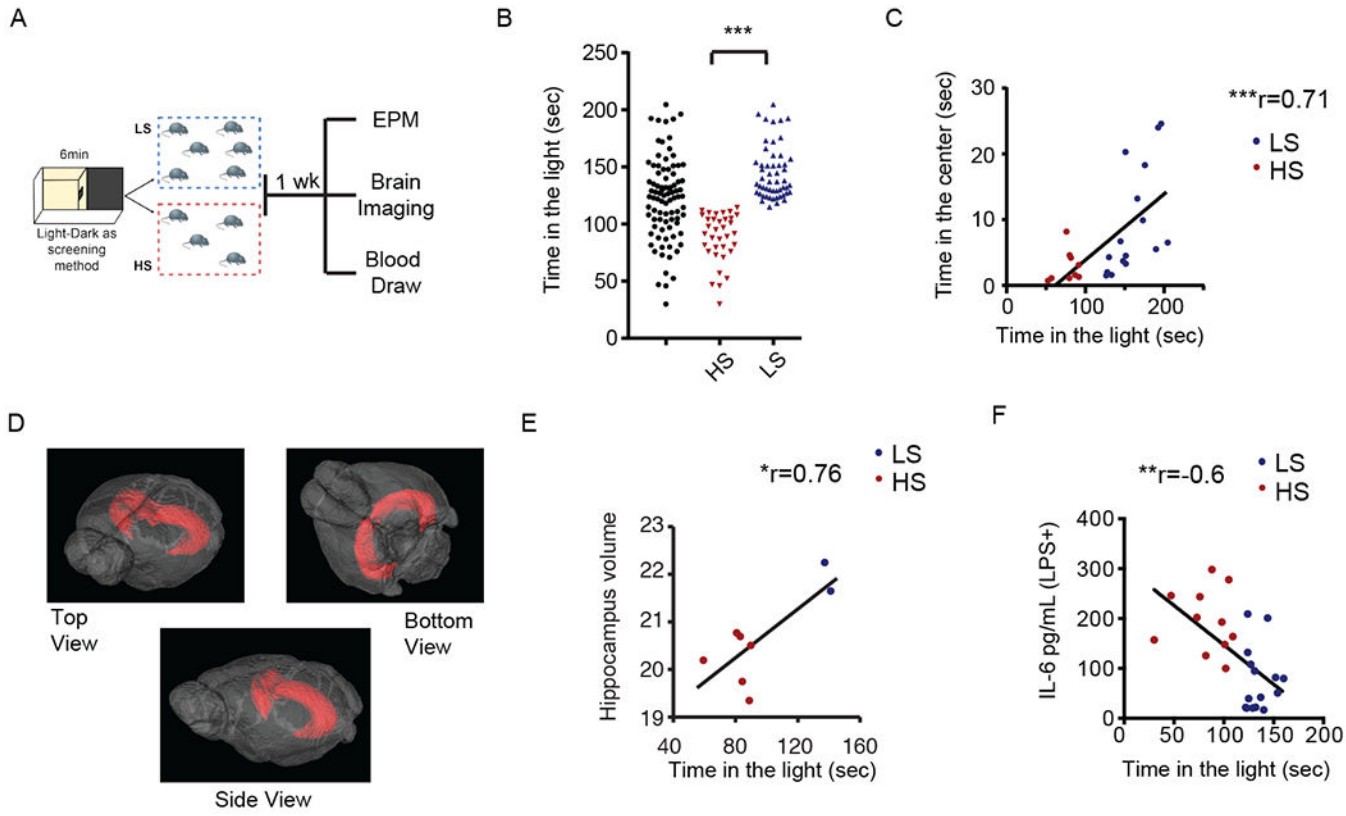

Figure 1. Interrelated biomarkers characterize HS and LS bio-behavioral phenotypes.

(A) Schema of the experimental design to identify HS and LS phenotypes. **(B)** Mice designated as high susceptible (HS, n=35) at the light dark screening spent less time in the light chamber as compared to mice designated as low susceptible (LS, n=53). **(C)** Positive correlation between time in the light chamber and time in the center at the LDT, further supporting the occurrence of basal anxiety-like behavior in the HS phenotype (n=25) **(D)** Representative three-dimensional images of hippocampal volume (Top, bottom and side views). **(E)** Exploratory analyses showed a positive correlation between the time in the light chamber of the LDT and hippocampal volume whereby the smallest the hippocampal volume the lower was the time in the light chamber at the LDT (n=8). **(F)** Interrelated bio-behavioral measures distinguished the HS and LS phenotypes: mice designated as HS at the LDT screening also showed higher levels of IL-6 release when stimulated ex vivo with LPS as compared to LS mice, whereby the highest the levels of stimulated IL-6 the lowest was the time in the light chamber at the LDT. Asterisk indicates significant comparisons with corresponding controls, *p<0.05, **p<0.01, ***p<0.001 at Student's two-tailed t test or Spearman test. See also SI Figures 1 and 2.

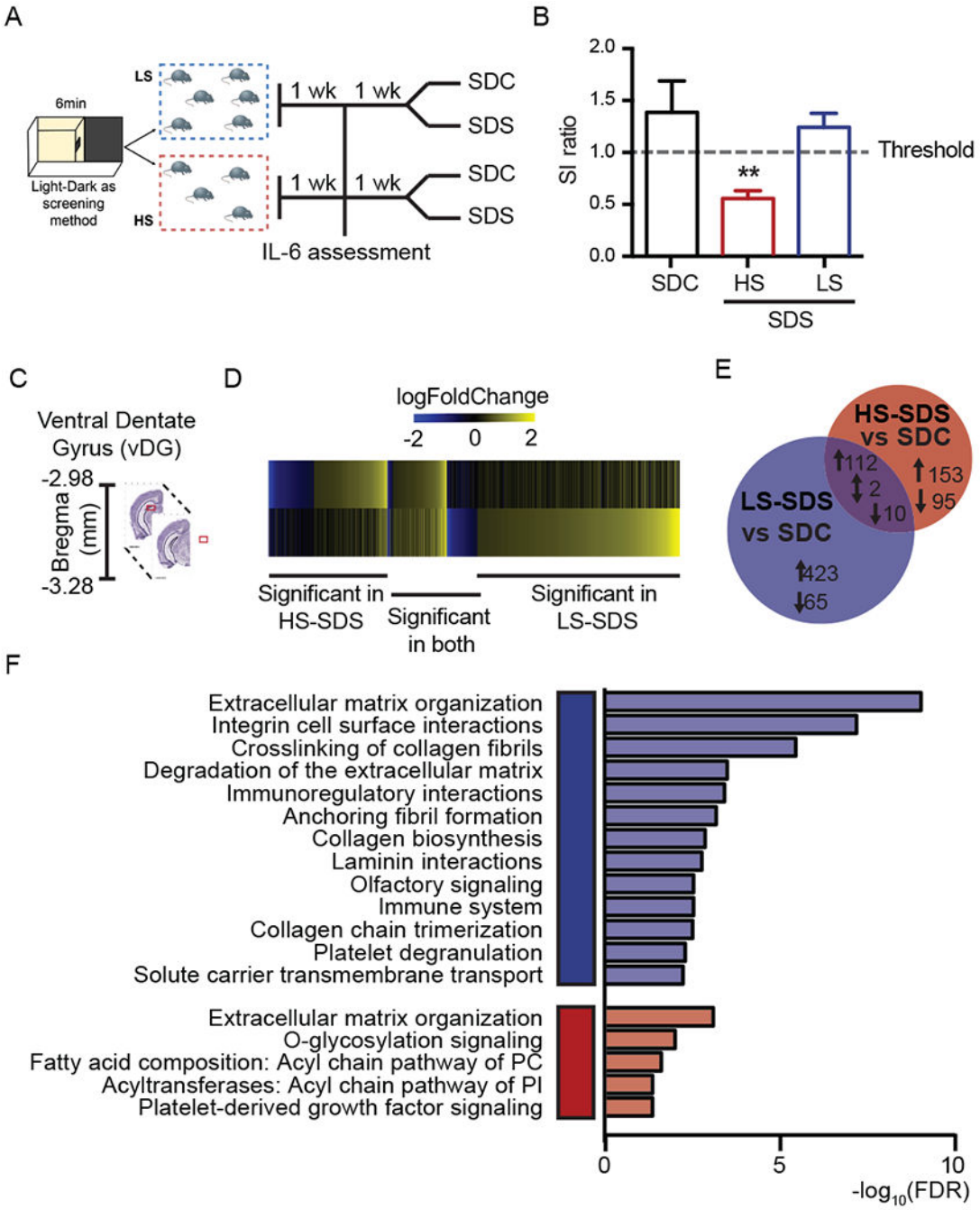

Figure 2. Behavioral responses and transcriptome-wide changes in the ventral dentate gyrus after social defeat stress in the HS and LS phenotypes.

(A) Schema of the experimental design employed to study the effects of social defeat stress on the HS and LS phenotypes. **(B)** The HS phenotype showed social withdrawal after SDS as compared to unstressed control mice and the LS phenotype. Specifically, HS mice showed decreased social interaction 24 hours after the last defeat episode as compared with unstressed control mice and LS mice (N per study group: Ctrl=9, HS=21, LS=15; one-way ANOVA $F_{2,42}$=4; p=0.0002). **(C)** Representative coronal brain images with references to the

ventral dentate gyrus (vDG, highlighted in red) used for brain microdissection. **(D)** Heatmap of SDS-regulated expression changes in HS and LS phenoytypes as compared to unstressed control mice. **(E)** SDS altered transcriptional expression of 372 genes (fold change >1.3) in HS mice as compared to the 612 genes altered in LS mice with 124 overlapping gene changes in HS and LS mice. **(F)** Enrichment pathway analyses showed that SDS differentially affects several relevant signaling networks within the vDG of the HS versus LS phenotypes. Of note, SDS altered pathways related to acyltransferase and fatty acids composition in the HS phenotype. Bars represent mean ± SEM, and asterisk indicates significant comparisons, **$p<0.01$ at post-hoc analysis. See also SI Tables 1–3.

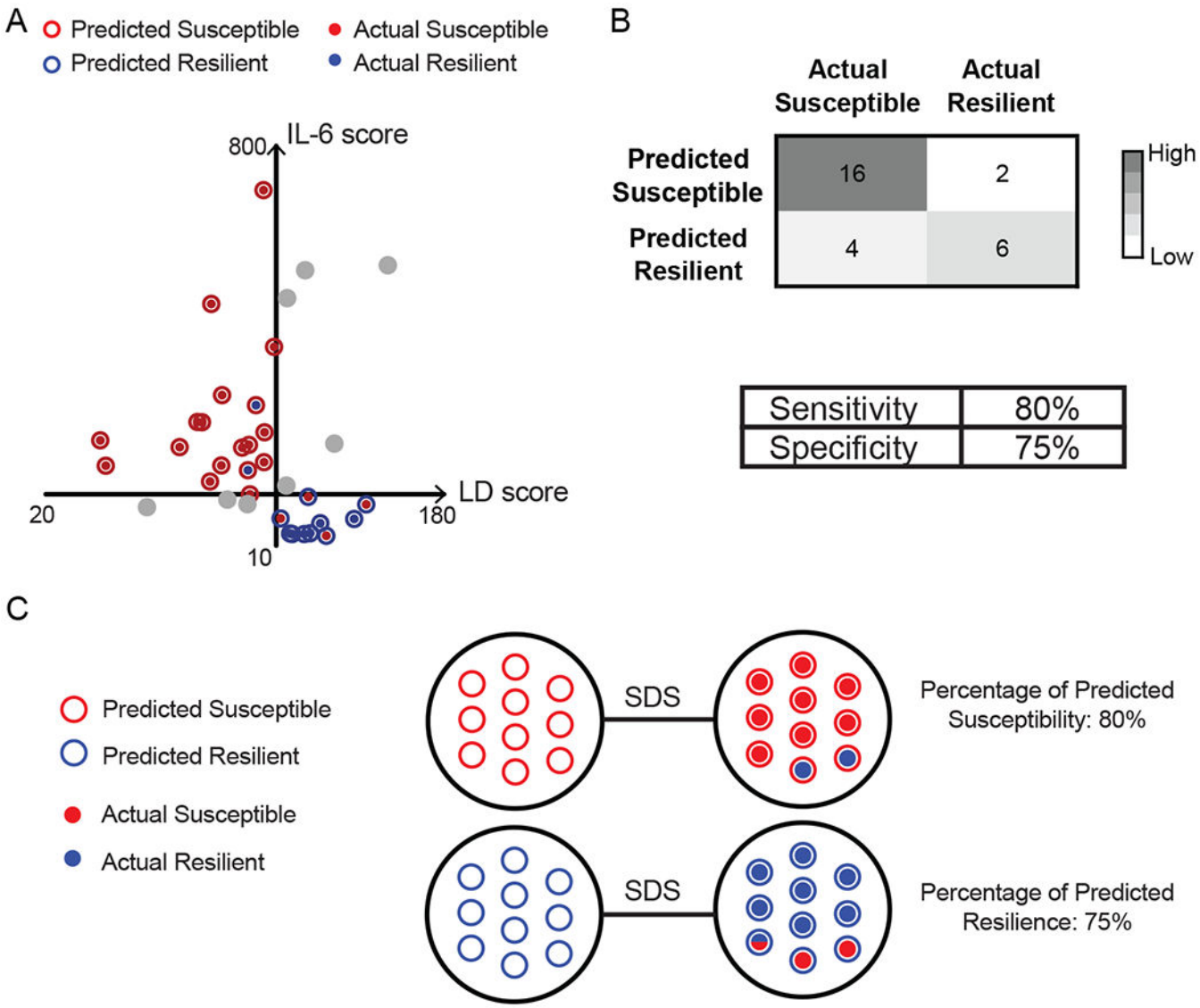

Figure 3. Multidimensional markers of susceptibility or resilience predict the behavioral responses to stress.

(A) Scatterplot for the predicted and observed susceptible and resilient mice along the dimensions of LD and IL-6 scores. Gray dots represent mice with uncertain classification. **(B)** Confusion matrix depicting the performance of the classifier in predicting susceptible and resilient mice at the social interaction test on the basis of integrated measures of LD and IL-6 scores (i.e., time in the light chamber at the LTD and stimulated IL-6 levels before any applied stress). **(C)** A schematic depicting the predictive ability of the classifier based upon the identified in-vivo biomarkers of susceptibility versus resilience to stress.

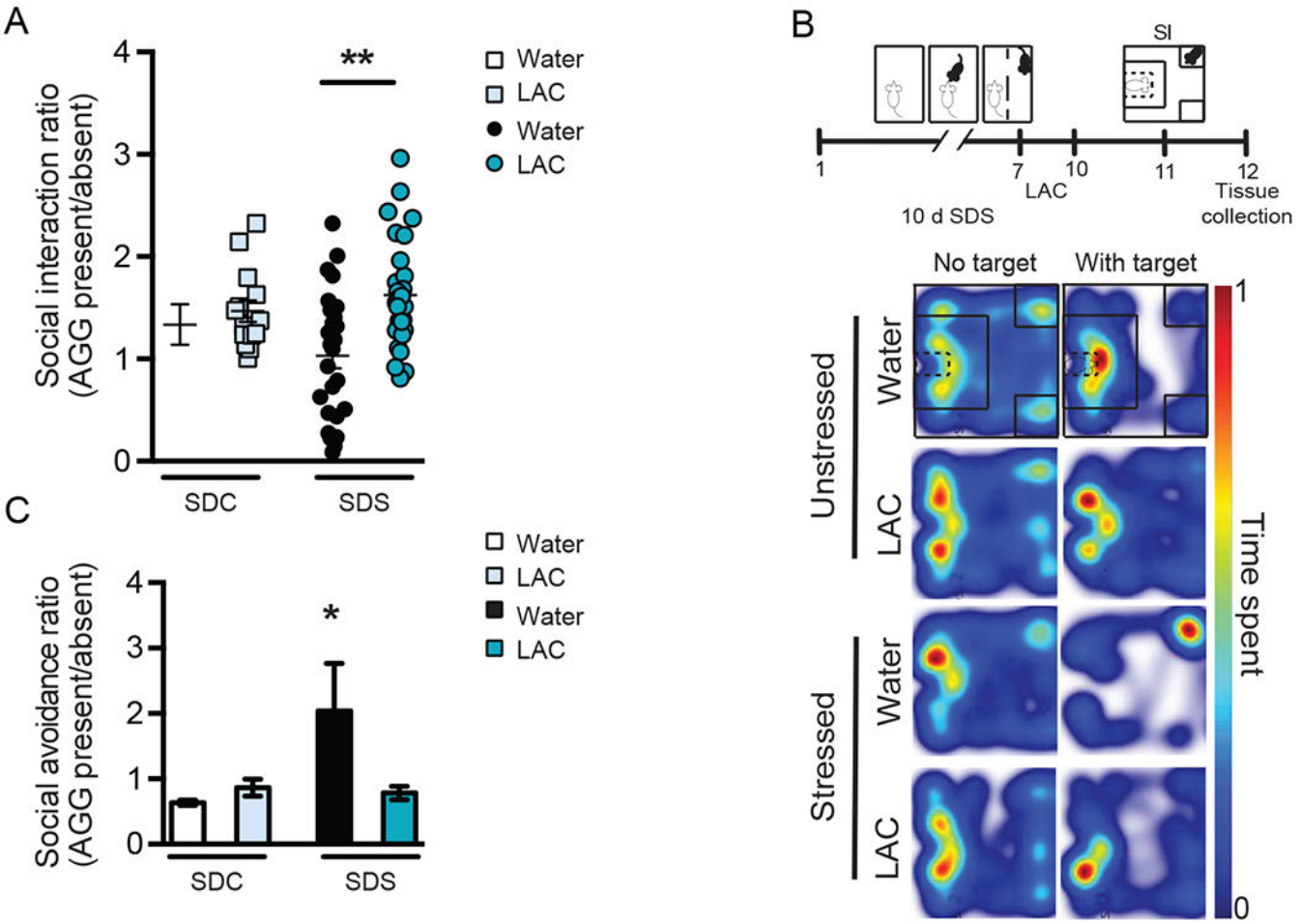

Figure 4. Effects of Acetyl-L-carnitine (LAC) at the SDS paradigm.

(A-B) SDS mice after receiving LAC administration showed a social interaction ratio similar to the levels of unstressed control mice and significantly different from the social interaction ratio of SDS mice receiving vehicle. *B* shows representative behavioral heatmaps (two-way ANOVA [treatment] $F_{1,52}$=4.03, p=0.05; [stress] $F_{1,52}$=2.47, p=0.12; [interaction] $F_{1,52}$=6.82, p=0.001; N per study group: unstressed water: 11, stressed LAC: 14, stressed water: 26, stressed LAC: 29). **(C)** Administration of LAC in SDS mice improved social avoidance (two-way ANOVA [treatment] $F_{1,52}$=3.1, p=0.08; [stress] $F_{1,52}$=5.14, p=0.03; [interaction] $F_{1,52}$=6.5, p=0.01). Bars represent mean ± SEM, and asterisk indicates significant comparisons with corresponding controls, *p<0.05, **p<0.01 at Student's two-tailed t test.

KEY RESOURCES TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use “this paper” if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources.	Include any additional information or notes if necessary.
Antibody	CD11b (clone M1/70)			
Antibody	CSF-1R (also called CD115) (clone AFS98)			
Antibody	Ly6C (clone HK1.4)			
Antibody	Ly6G (clone 1A8)			
Secondary Reagents	allophycocyanin, peridinine chlorophyll protein, and phycoerythrin-indotricarbocyanine-conjugated streptavidin			
Biological Sample	Blood, C57BL/6, male	this paper		
Biological Sample	Whole brain, C57BL/6, male	this paper		
Biological Sample	Ventral dentate gyrus, C57BL/6, male	this paper		
Chemical Compound or Drug	Acetyl-L-carnitine	Sigma Aldrich	A6706	
Commercial Assay Or Kit	IL-6 ELISA	BD Biosciences	550950	
Commercial Assay Or Kit	Truseq mRNA library prep kit	Illumina	RS-122-2001/2	
Deposited Data; Public Database	RNAseq, GEO ID in progress	NCBI GEO DataSets	RRID:SCR_005012; https://www.ncbi.nlm.nih.gov/gds	
Organism/Strain	Mouse: C57BL/6, male	Charles River		
Software; Algorithm	R version 3.4.4			
Software; Algorithm	GraphPad Prism 6			
Software; Algorithm	MAGeT algorithm	Chakravarty et al, 2013		
Software; Algorithm	FastQC	http://www.bioinformatics.babraham.ac.uk/projects/fastqc		
Software; Algorithm	Trimmomatic	Bolger et al., 2014		
Software; Algorithm	TopHat	Kim et al., 2013		
Software; Algorithm	CuffDiff	Trapnell et al, 2012		