Archival Report

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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 1) integrative brain-body behavioral, immunological, and structural domains characterized and predicted susceptibility or resilience to social defeat stress (SDS) in mice and 2) administration of acetyl-L-carnitine promoted resilience at the SDS paradigm.

RESULTS: Our findings identified multidimensional brain-body predictors of susceptibility versus resilience to SDS. The copresence 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 the ventral dentate gyrus after SDS. At the individual level, a computational approach predicted whether 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 acetyl-L-carnitine 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: Acetylcarnitine, Biomarkers, Epigenetic, Individual differences, Phenotype, Risk factors

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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 that predispose apparently healthy individuals to cope with stress or develop the deleterious effects of stress on brain and systemic functions.

Prediction of individuals at risk of developing stress-induced disorders has been based largely on single risk factors. For example, previous work identified increased anxiety-like behavior resulting from glucocorticoid overactivation as a risk factor for development of stress-induced glutamatergic dysfunction in the ventral hippocampus and 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 ventral hippocampus (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 proresilient actions of the glutamatergic agent ketamine at the

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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 (10,20–27). 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 2 (mGluR2) and corresponding structural plasticity. mGluR2 is a key inhibitor of spontaneous glutamate release. However, potential proresilient actions of LAC remain to be elucidated.

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

METHODS AND MATERIALS

More information is available in Supplement 1.

Behavioral Assessment Prior to or After SDS

The light-dark test (LDT) as the screening method for individual susceptibility was performed as previously described (8) and is detailed in Supplement 1.

One week after the LDT screening, mice were tested in the elevated plus maze as previously described (28) (more details in Supplement 1).

The social interaction test was performed at the end of the SDS paradigm as previously described (29) (more details in Supplement 1).

Immunological Assessment

Flow Cytometry. Flow cytometry studies were performed using an LSRII Fortessa (Becton Dickinson, Franklin Lakes, NJ) and analyzed using FlowJo software (Tree Star, Ashland, OR). Fluorochrome or biotin-conjugated monoclonal antibodies 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, peridinin chlorophyll protein, and phycoerythrin-indotricarbocyanine-conjugated streptavidin) were obtained from BD Biosciences (San Jose, CA), eBioscience (San Diego, CA), or BioLegend30 (San Diego, CA).

Leukocyte Isolations/Immune Challenge. Whole blood (200 μL) was transferred to a 15-mL conical tube and mixed with 2 mL of complete media (20% horse serum,10% fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin). The blood/media mixture was layered over an equal volume of Ficoll-Paque Plus (GE Healthcare, Chicago, IL). Samples were centrifuged (790*g*, 15 minutes, 25°C) to form a buffy coat layer. Cells were removed, washed in BEP solution (phosphate-buffered saline with 0.5% bovine serum albumin, and 2 mM ethylenediamine tetraacetate), and centrifuged (529*g*, 8 minutes, 25°C). The supernatant was removed and cells were resuspended 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-Aldrich, St. Louis, MO) and stored for 24 hours at 37°C with 5% CO_2. After 24 hours, cells and media were removed from plates and centrifuged (2348*g*, 5 minutes), and the supernatant was removed and stored at −80°C until IL-6 analysis.

IL-6 enzyme-linked immunosorbent assay was performed as previously described (7).

Brain Imaging and Ex Vivo Magnetic Resonance Imaging Scans

One week after LDT testing, brains were processed for brain imaging as previously described (30).

Stress Paradigm

SDS was performed as previously described (29).

Pharmacological Approach

LAC (Sigma-Aldrich) 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 a control. Mice were administered either the vehicle or LAC solution treatment until behavioral testing. Reports from previous studies (10,21) were used to ensure 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 RNA sequencing gene expression and bioinformatics analysis was performed as previously described (10,31). Significance was set at uncorrected $p < .05$ for broad pattern identification, and fold-change threshold was set at ±30%. Significance was set at false discovery rate <.05 for pathway analysis by using the Reactome Pathways in Panther (32,33). More details can be found in Supplement 1.

Computational Approach to Classify Susceptible and Resilient Phenotypes

We used a computational approach in R (R Foundation for Statistical Computing, version 3.4.4, Vienna, Austria) 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 100 pg/mL for the LDT and IL-6 scores, respectively (7,8), were used to classify mice predicted to become susceptible (i.e., LDT score <115 and IL-6 score >100) or remain resilient (i.e., LDT score >115 and IL-6 score <100). Mice with LDT score <115 and IL-6 score <100 or LDT 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.

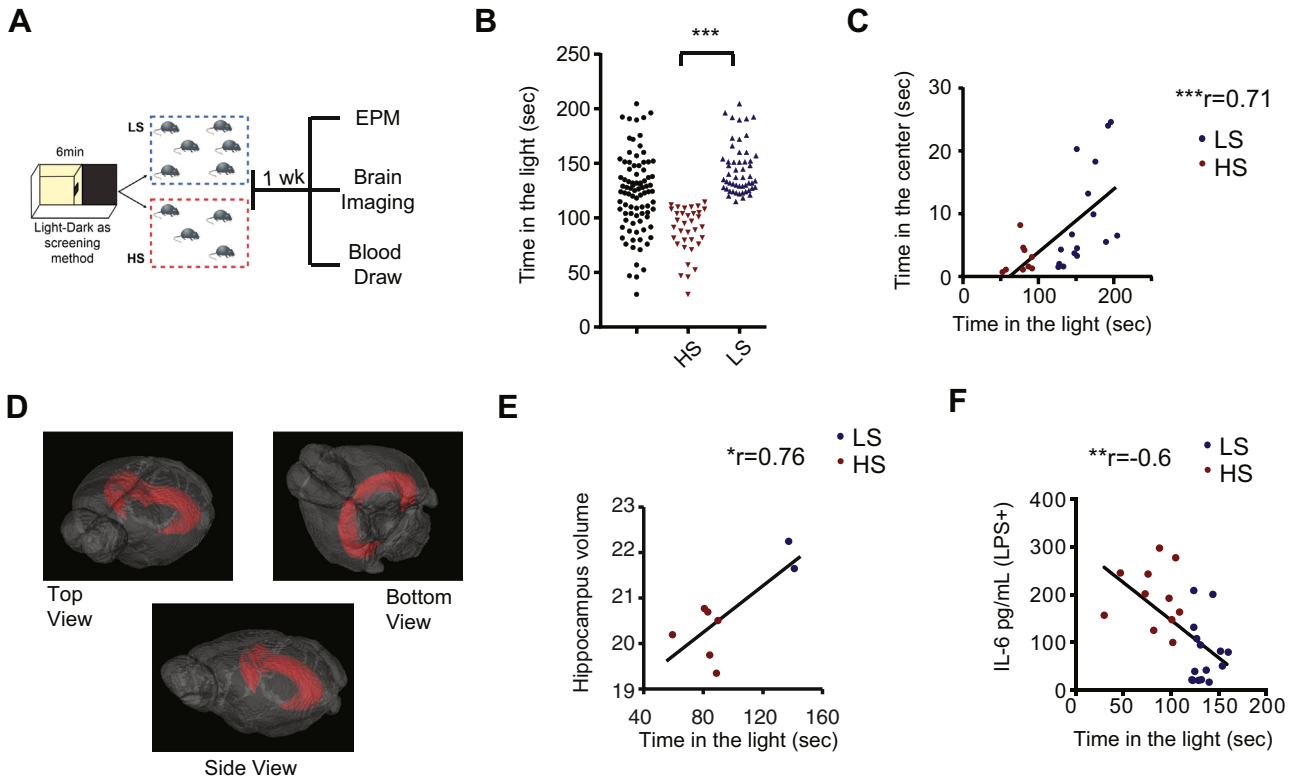

Figure 1. Interrelated biomarkers characterize high-susceptible (HS) and low-susceptible (LS) biobehavioral phenotypes. **(A)** Schema of the experimental design to identify HS and LS phenotypes. **(B)** Mice designated as HS (n = 35) at the light-dark screening spent less time in the light chamber as compared with mice designated as LS (n = 53). **(C)** Positive correlation between time in the light chamber and time in the center at the light-dark test (LDT), further supporting the occurrence of basal anxiety-like behavior in the HS phenotype (n = 25). **(D)** Representative 3-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 smaller the hippocampal volume, the less time in the light chamber at the LDT (n = 8). **(F)** Interrelated biobehavioral measures distinguished the HS and LS phenotypes: mice designated as HS at the LDT screening also showed higher levels of interleukin (IL)-6 release when stimulated ex vivo with lipopolysaccharide (LPS) as compared with LS mice, whereby the higher the levels of stimulated IL-6, the less time in the light chamber at the LDT. Asterisks indicate significant comparisons with corresponding control animals; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ at Student's 2-tailed t test or Spearman test. See also Figures S1 and S2 in Supplement 1. EPM, elevated plus maze.

Statistics

Statistical analyses were performed using 1- or 2-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, or 2-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 High-Susceptible and Low-Susceptible Biobehavioral Phenotypes

We recently introduced a modified version of the 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 before any applied stress. This subset of mice showed a decrease in mGluR2 expression in the hippocampus with corresponding depressive-like behavior after exposure to chronic restraint stress (8,21,34). Here, we used the LDT (Figure 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, which 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 (Figure 1B), also spent less time in the center of the open chamber as compared with mice designated as low susceptible (LS) (Figure 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 as compared with LS mice (Figure S1A, B in Supplement 1). 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 (8,35). 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

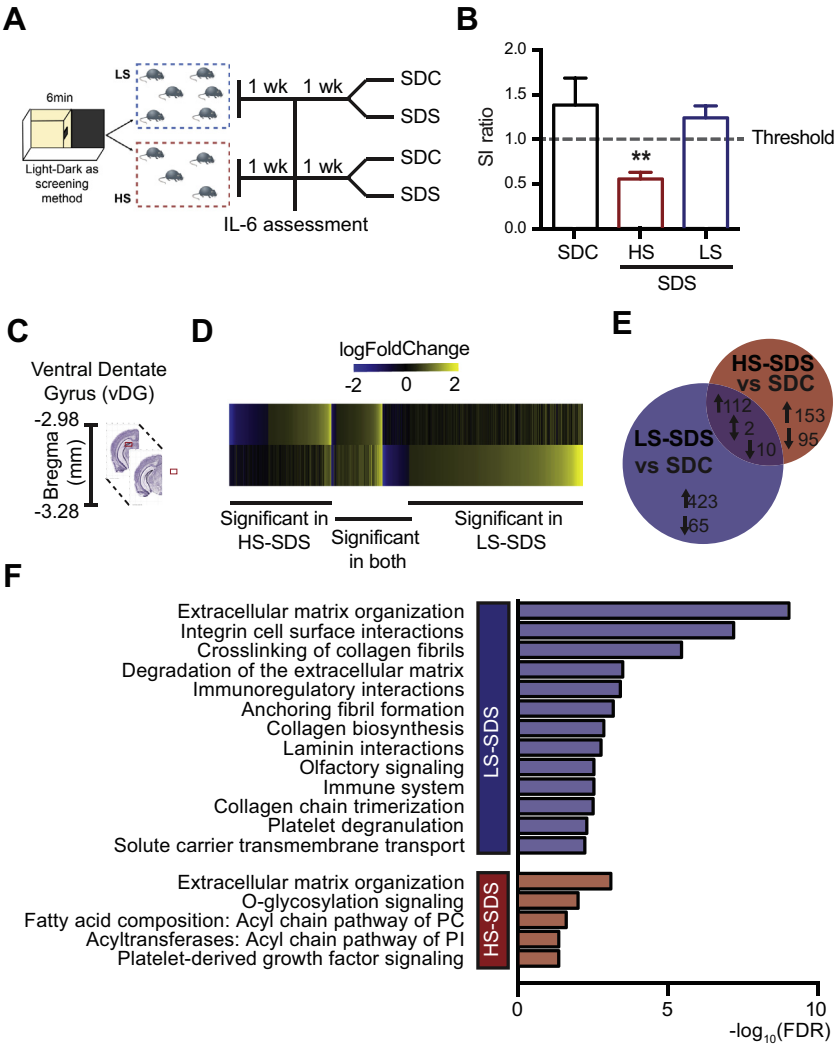

Figure 2. Behavioral responses and transcriptome-wide changes in the ventral dentate gyrus after social defeat stress (SDS) in the high-susceptible (HS) and low-susceptible (LS) phenotypes. **(A)** Schema of the experimental design employed to study the effects of SDS on the HS and LS phenotypes. **(B)** The HS phenotype showed social withdrawal after SDS as compared with unstressed control mice and the LS phenotype. Specifically, HS mice showed decreased social interaction (SI) 24 hours after the last defeat episode as compared with unstressed control mice and LS mice (n per study group: control group = 9, HS = 21, LS = 15; 1-way analysis of variance [$F_{2,42} = 4$, $p = .0002$]). **(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 with unstressed control mice. **(E)** SDS altered transcriptional expression of 372 genes (fold change >1.3) in HS mice as compared with 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 vs. LS phenotypes. Of note, SDS altered pathways related to acyltransferase and fatty acids composition in the HS phenotype. Bars represent mean ± SEM, and asterisks indicate significant comparisons; $^{**}p < .01$ at post hoc analysis. See also Tables S1–S3 in Supplement 2. FDR, false discovery rate; IL-6, interleukin-6; PC, phosphatidylcholine; PI, phosphatidylinositol; SDC, social defeat control (unstressed group).

the highest anxiety-like behavior had the smallest hippocampal volume prior to any applied stressor (Figure 1D, E).

To further characterize the HS and LS phenotypes, we examined several markers of the systemic immune system, including the proinflammatory 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 toward higher counts of inflammatory monocytes prior to any stress exposure (Figure S2A, B in Supplement 1). 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 (Figure 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 leukocyte population or IL-6 without LPS stimulation were detected (Figure S2C, D in Supplement 1). Together, these data show distinct biobehavioral phenotypes of the HS and LS mice.

HS and LS Phenotypes Modulate Susceptibility and Resilience to SDS

First, we tested whether the HS and LS biobehavioral phenotypes could modulate the behavioral responses to stress. To test this hypothesis, we subjected both HS and LS mice to 10 days of SDS (Figure 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 (Figure 2B). These data show that the HS and LS biobehavioral 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 to capture transcriptome-wide alterations in HS and LS mice after exposure to SDS (HS-SDS and LS-SDS, respectively) as compared with the unstressed control group. We narrowed our sequencing approach

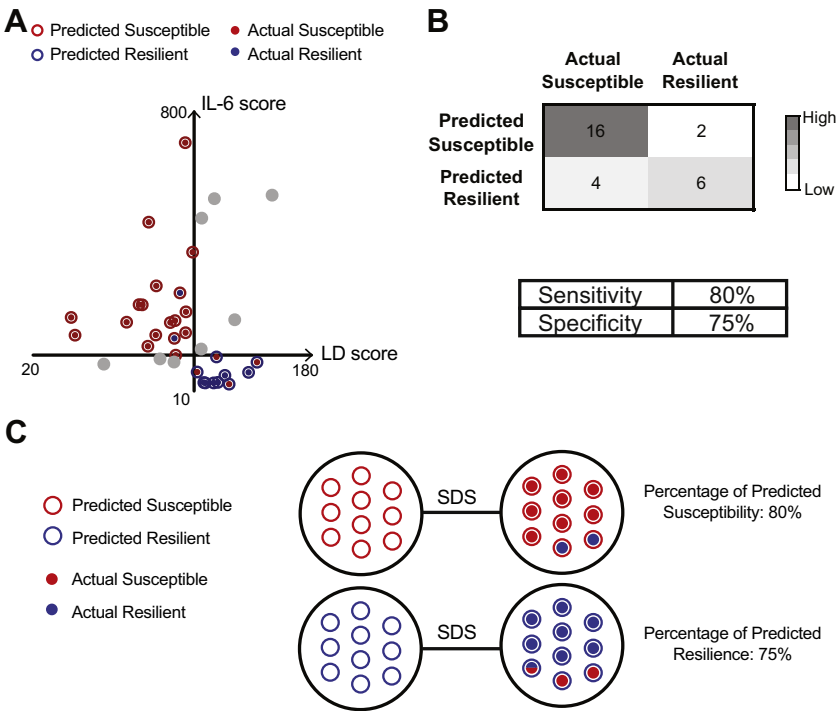

Figure 3. Multidimensional markers of susceptibility or resilience predict the behavioral responses to stress. **(A)** Scatterplot for the predicted and observed susceptible and resilient mice along the dimensions of light-dark (LD) test and interleukin (IL)-6 scores. Gray dots represent mice with uncertain 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 test and IL-6 scores (i.e., time in the light chamber at the LD test and stimulated IL-6 levels before any applied stress). **(C)** A schematic depicting the predictive ability of the classifier based on the identified in vivo biomarkers of susceptibility vs. resilience to stress. SDS, social defeat stress.

to the ventral dentate gyrus (Figure 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, $p < .05$) in HS mice compared with the 612 genes in LS mice, with 124 overlapping gene changes between HS-SDS and LS-SDS mice (Figure 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 10 genes that were selectively altered in HS-SDS mice but not in LS-SDS mice (Table S1 in Supplement 2). *ApoC3* is a gene involved in the maintenance of homeostasis of triglycerides.

Therefore, our data show that the HS phenotype, which is characterized by copresence of anxiety, elevated leukocyte-derived IL-6, and small hippocampal volume before any applied stressor became later susceptible to SDS, manifested 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 whether a given animal will develop SDS-induced social withdrawal or remain 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 100 pg/mL for the LDT and IL-6 scores, respectively to classify mice predicted to become susceptible (i.e., LDT score <115 and IL-6 score >100) or remain resilient (i.e., LDT score >115 and IL-6 score <100) (Figure 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 Figure 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 of the 18 predicted susceptible mice manifested social withdrawal, whereas 2 did not (Figure 3B). With regard to the prediction of resilience to SDS, 6 of the 10 predicted resilient mice were actual resilient, whereas 4 developed social withdrawal (Figure 3B). To quantify the estimated probabilities that the predicted phenotypes reflected the actual numbers of mice exhibiting or not exhibiting 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) (Figure 3B). Next, we tested the strength of the classifier versus

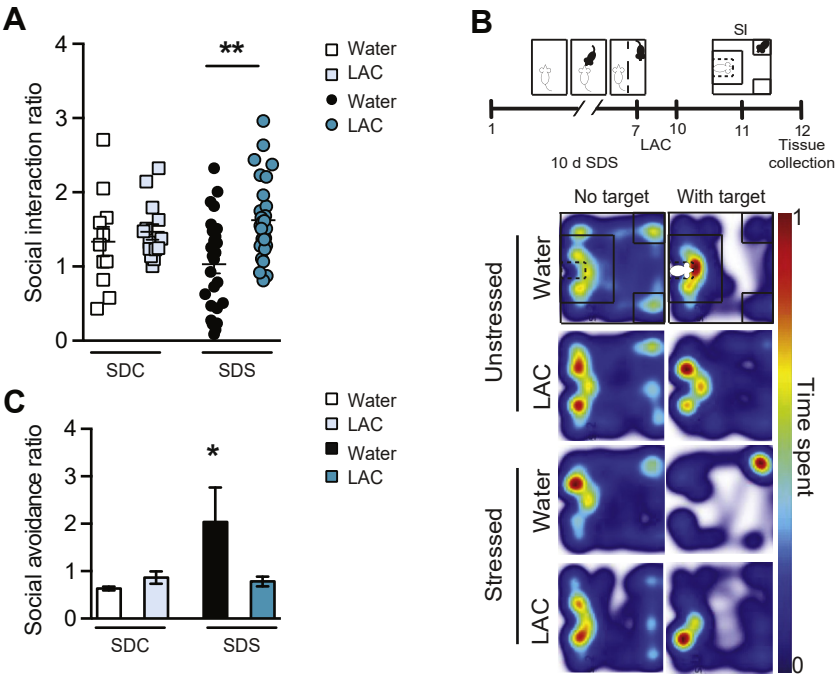

Figure 4. Effects of acetyl-L-carnitine (LAC) at the social defeat stress (SDS) paradigm. **(A, B)** SDS mice after receiving LAC administration showed a social interaction (SI) ratio similar to the levels of unstressed control mice (social defeat control [SDC]) and significantly different from the SI ratio of SDS mice receiving vehicle. **(B)** Representative behavioral heatmaps (2-way analysis of variance: treatment [$F_{1,52} = 4.03$, $p = .05$], stress [$F_{1,52} = 2.47$, $p = .12$], interaction [$F_{1,52} = 6.82$, $p = .001$]; 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 (2-way analysis of variance: treatment [$F_{1,52} = 3.1$, $p = .08$], stress [$F_{1,52} = 5.14$, $p = .03$], interaction [$F_{1,52} = 6.5$, $p = .01$]). Bars represent mean ± SEM, and asterisks indicate significant comparisons with corresponding control mice; $^{*}p < .05$, $^{**}p < .01$ at Student's 2-tailed t test.

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 each individual measure alone, as shown by a higher sensitivity of 80% as compared with 76% for the LDT categorization alone (Figure S3A in Supplement 1) and 72% for the IL-6 categorization alone (Figure S3B in Supplement 1). We reason that each individual measure miscategorizes some mice that instead were classified as "uncertain" by the combined measures (gray dots in Figure 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 Proresilient Effects of 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 that the findings above from the RNA sequencing 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 (Figure 4B) led to proresilient 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 traveled in the social interaction test (Figure S4 in Supplement 1). 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 levels of unstressed control mice (Figure 4A, B) and were significantly different from stressed mice that received vehicle (Figure 4A, B). Likewise, SDS mice that received administration of LAC showed a decrease in social avoidance ratio to the levels of unstressed control mice (Figure 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 proresilient effects of the epigenetic modulator of glutamatergic function 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 mechanisms 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 (3,5,36), 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 hypothalamic-pituitary-adrenal 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 at investigating whether potential preexisting 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 biobehavioral 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 apparently healthy individuals to develop (or protecting them from) 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 proresilient responses to administration of LAC were seen after just a few days of administration at the SDS paradigm (10,20–22). 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 mGluR2s in the ventral dentate gyrus.

Reinforcing the importance of brain-body communication, LAC also ameliorates insulin resistance (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 (37). Thus, it is expected that other mechanisms that result from lack of central and systemic resilience (17,38) may be implicated in the mechanism of action of LAC. One attractive hypothesis for future research on determining possible mechanistic targets in the proresilient action of LAC is that administration of LAC exerts proinflammatory effects by decreasing IL-6 levels in susceptible phenotype to promote successful adaptation to stress.

Future studies are also needed to further characterize the HS and LS phenotypes identified here as well as to investigate epigenetic/environmental factors early in life that determine the origin of the distinct biobehavioral phenotypes. Given that 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 (39,40). 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 (41). Recent studies showed that early life stress encodes lifelong susceptibility to SDS (31). 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 major depressive disorder (42). Recently, we reported decreased LAC levels in two independent populations of patients suffering from major depressive disorder (27,42). 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 (27,42). Exposure to childhood trauma has also been linked to inflammatory states, such as insulin resistance (43), a metabolic dysfunction associated with both LAC deficiency and decreased hippocampal volume (44). This knowledge will also inform treatment decision. If an 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 (45).

In conclusion, the current findings of a biobehavioral 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 proresilient 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.

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ARTICLE INFORMATION

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