Distinct Proteomic, Transcriptomic, and Epigenetic Stress Responses in Dorsal and Ventral Hippocampus

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Abstract: BACKGROUND Acutely stressful experiences can trigger neuropsychiatric disorders and impair cognitive processes by altering hippocampal function. Although the intrinsic organization of the hippocampus is highly conserved throughout its long dorsal-ventral axis, the dorsal (anterior) hippocampus mediates spatial navigation and memory formation, whereas the ventral (posterior) hippocampus is involved in emotion regulation. To understand the molecular consequences of stress, detailed genome-wide screens are necessary and need to distinguish between dorsal and ventral hippocampal regions. While transcriptomic screens have become a mainstay in basic and clinical research, proteomic methods are rapidly evolving and hold even greater promise to reveal biologically and clinically relevant biomarkers. METHODS Here, we provide the first combined transcriptomic (RNA sequencing) and proteomic (sequential window acquisition of all theoretical mass spectra [SWATH-MS]) profiling of dorsal and ventral hippocampus in mice. We used three different acute stressors (novelty, swim, and restraint) to assess the impact of stress on both regions. RESULTS We demonstrated that both hippocampal regions display radically distinct molecular responses and that the ventral hippocampus is particularly sensitive to the effects of stress. Separately analyzing these structures greatly increased the sensitivity to detect stress-induced changes. For example, protein interaction cluster analyses revealed a stress-responsive epigenetic network around histone demethylase Kdm6b restricted to the ventral hippocampus, and acute stress reduced methylation of its enzymatic target H3K27me3. Selective Kdm6b knockdown in the ventral hippocampus led to behavioral hyperactivity/hyperresponsiveness. CONCLUSIONS These findings underscore the importance of considering dorsal and ventral hippocampus separately when conducting high-throughput molecular analyses, which has important implications for fundamental research as well as clinical studies.

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Distinct Proteomic, Transcriptomic, and Epigenetic Stress Responses in Dorsal and Ventral Hippocampus

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ABSTRACT

BACKGROUND: Acutely stressful experiences can trigger neuropsychiatric disorders and impair cognitive processes by altering hippocampal function. Although the intrinsic organization of the hippocampus is highly conserved throughout its long dorsal-ventral axis, the dorsal (anterior) hippocampus mediates spatial navigation and memory formation, whereas the ventral (posterior) hippocampus is involved in emotion regulation. To understand the molecular consequences of stress, detailed genome-wide screens are necessary and need to distinguish between dorsal and ventral hippocampal regions. While transcriptomic screens have become a mainstay in basic and clinical research, proteomic methods are rapidly evolving and hold even greater promise to reveal biologically and clinically relevant biomarkers.

METHODS: Here, we provide the first combined transcriptomic (RNA sequencing) and proteomic (sequential window acquisition of all theoretical mass spectra [SWATH-MS]) profiling of dorsal and ventral hippocampus in mice. We used three different acute stressors (novelty, swim, and restraint) to assess the impact of stress on both regions.

RESULTS: We demonstrated that both hippocampal regions display radically distinct molecular responses and that the ventral hippocampus is particularly sensitive to the effects of stress. Separately analyzing these structures greatly increased the sensitivity to detect stress-induced changes. For example, protein interaction cluster analyses revealed a stress-responsive epigenetic network around histone demethylase *Kdm6b* restricted to the ventral hippocampus, and acute stress reduced methylation of its enzymatic target H3K27me3. Selective *Kdm6b* knockdown in the ventral hippocampus led to behavioral hyperactivity/hyperresponsiveness.

CONCLUSIONS: These findings underscore the importance of considering dorsal and ventral hippocampus separately when conducting high-throughput molecular analyses, which has important implications for fundamental research as well as clinical studies.

Keywords: Dorsal, Epigenetics, H3K27me3, Hippocampus, *Kdm6b*, Proteomics, Sequencing, Stress, SWATH-MS, Transcriptomics, Ventral

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Acutely stressful life events can precipitate mood and anxiety disorders, posttraumatic stress disorder, and cognitive dysfunction in humans (1–4). A key target of the acute stress response is the hippocampus (5–7), which has a highly conserved intrinsic organization, but its connectivity to other brain regions is strikingly different along its longitudinal axis (dorsal to ventral in rodents, posterior to anterior in humans) (8,9). Functionally, the dorsal hippocampus (dHC) is critical for learning and memory performance, while the ventral hippocampus (vHC) is involved in anxiety and behavioral inhibition (10–13). These vast functional differences are also reflected in the transcriptomic profile of dHC and vHC (14–16). Stress can modulate hippocampal plasticity in opposite directions along the longitudinal axis, impairing long-term potentiation in the dHC and enhancing it in the vHC (17–19). Similarly, a few studies have reported strikingly different effects of stress in dHC versus vHC at the level of morphologic changes (20), epigenetic regulation (21,22), and gene and protein expression (19,23). RNA sequencing has recently revealed specific transcriptomic effects of chronic stress in the vHC and led to the identification of functionally relevant molecular targets (24,25). However, in comparison with chronic stress, the molecular impact of acute stress remains poorly characterized (26). Transcriptomic analyses after acute stress have so far studied only whole hippocampal tissue (27–30). Therefore, we use RNA sequencing and state-of-the-art sequential window acquisition of all theoretical mass spectra (SWATH-MS) proteomics to provide the first genome-wide characterization of gene and protein differences between dHC and vHC. We demonstrate that dHC and vHC differ profoundly in their molecular

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composition and that they respond to acute stressors in strikingly different ways, with unexpectedly unique transcriptional profiles triggered by each stressor.

METHODS AND MATERIALS

Animals

C57BL/6J male mice (2.5 months old) were obtained from Janvier Labs (Saint Berthevin, France) and maintained in a temperature- and humidity-controlled facility on a 12-hour reversed light/dark cycle (lights off: 8:15 AM; lights on: 8:15 PM). All procedures were carried out in accordance with Swiss cantonal regulations for animal experimentation and were approved under license 155/2015.

Stress Paradigms

For all experiments, mice were single housed 24 hours before stress/handling/testing (31). For novelty stress, each mouse was placed in the center of a square box for 6 minutes. For restraint stress, each mouse was placed in a 50-mL Falcon tube with a large air hole cut out for its nose for 30 minutes. For cold swim stress, mice were placed in a plastic cylinder filled with 18 ± 1°C water for 6 minutes.

Tissue Processing

Mice were killed by cervical dislocation 45 minutes after the initiation of stress for transcriptomic analyses and 24 hours after initiation of stress for proteomic analyses (unless specified otherwise). The brain was immediately removed, and the hippocampus was dissected on ice and separated into dorsal and ventral halves using a razor blade. Tissue was snap frozen in liquid nitrogen and stored at −80°C until processing.

Statistics

Statistical analyses of proteomic and transcriptomic analyses are described in the corresponding sections. For all other analyses, two groups were compared using independent-samples *t* tests. For comparison of more than two groups, one-way analyses of variance were employed if there was a single factor. For two-factorial design (region × treatment), two-way analyses of variance were employed. Significant effects were analyzed using Tukey's post hoc tests. Data were routinely checked for outliers using Grubb's test. For all analyses, statistical significance was set to $p < .05$.

Proteomic Analyses. Data are available to readers via ProteomeXchange with identifier PXD006781. Samples were processed and analyzed using a previously described method (32). A detailed description of all steps is in Supplement 1.

RNA Sequencing Analyses. All data are available to readers via Gene Expression Omnibus with identifier GSE100236. Libraries were prepared using the TruSeq Stranded RNA Kit (Illumina, Inc., San Diego, CA) and single-end sequencing (Illumina HiSeq4000). A detailed description of all steps is in Supplement 1.

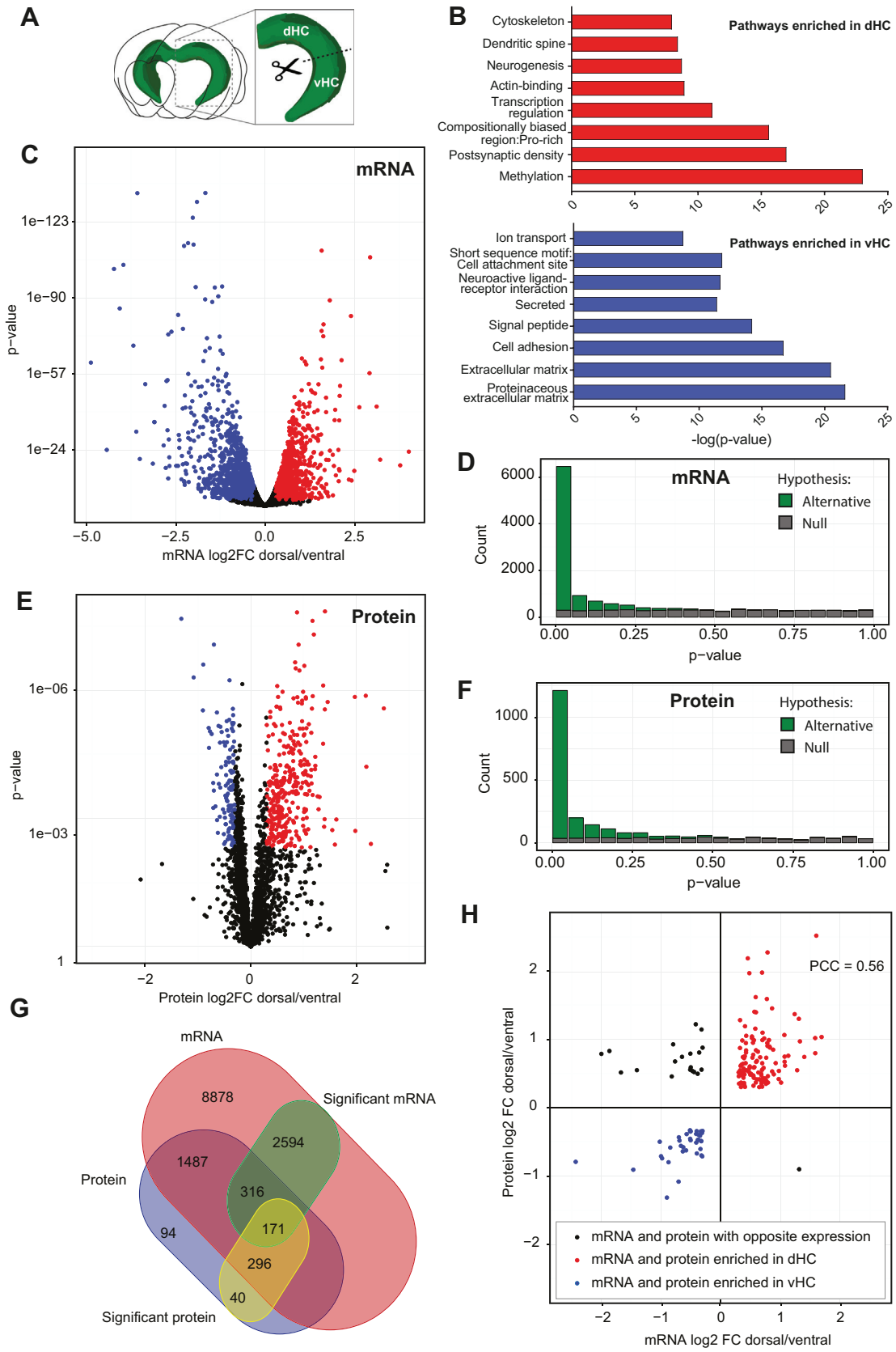
RESULTS

Transcriptomic Profiling of dHC and vHC

We dissected the hippocampus midway between the dorsal and ventral poles, as indicated in Figure 1A, and performed RNA sequencing on both dissections ($n = 5$ mice/group). Because gene expression differences are most profound between the dorsal and ventral poles of the hippocampus, with a gradual transition in the intermediary region (8,15), we expected that our approach would yield a conservative estimate of differences between dHC and vHC. We found that more than 20% of all detected genes (3081 of 13,902 genes) were differentially expressed between dHC and vHC ($p < .005$, log2 fold change $> \pm 0.3$) (Figure 1C); in total, 1639 genes were enriched in the dHC and 1442 were enriched in the vHC (Table S1 in Supplement 2). Functional enrichment analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) functional annotation tool (33) shows several strongly enriched categories specific for either dHC or vHC (Figure 1B). Previous transcriptomic work (15) had compared gene expression between dHC and vHC in principal neurons of different hippocampal subregions (CA1, CA3, and dentate gyrus). The authors identified 37 genes as differentially expressed between dHC and vHC across subregions, and we could validate 100% of these genes (Table S2 in Supplement 2). Furthermore, we could validate 84% of the 86 genes that Cembrowski *et al.* (15) found to differ in only one subregion of the hippocampus (Table S3 in Supplement 2). This shows that despite collecting whole tissue from dHC or vHC, our approach is sensitive enough to resolve subregion-specific effects restricted to a single hippocampal cell class. Altogether, we identified 490 genes with very strong expression differences (> twofold change, $p < .005$) between dHC and vHC (Table S4 in Supplement 2).

To gain insights into functional interactions between the genes enriched in either dHC or vHC, we created interaction networks based on known and predicted protein interaction networks (34) (for details, see Supplement 1). Genes enriched in dHC or vHC form distinct clusters (Figures S1 and S2 in Supplement 1; Tables S5 and S6 in Supplement 2). Pathway analyses on the top two clusters reveal enrichment for glutamatergic synapse, long-term potentiation, endocytosis, and regulation of actin cytoskeleton in the dHC and for neuroactive ligand-receptor activation, serotonergic synapse, and gamma-aminobutyric acidergic synapse in the vHC.

Figure 1. Transcriptomic and proteomic differences between dorsal hippocampus (dHC) and ventral hippocampus (vHC). **(A)** Schematic illustration of hippocampal dissection. **(B)** Pathway analysis for transcriptomic enrichment in dHC vs. vHC. **(C)** Transcriptomic results for messenger RNA (mRNA) enrichment in dHC (red) or vHC (blue). **(D)** The *p*-value distribution for transcriptomic analysis. **(E)** Proteomic results for protein enrichment in dHC (red) and vHC (blue). **(F)** The *p*-value distribution for proteomic analysis. **(G)** Venn diagram showing all identified, as well as all significantly enriched, mRNAs and proteins and their overlap. **(H)** Correlation between mRNAs and proteins enriched in dHC or vHC. FC, fold change; PCC, Pearson correlation coefficient.


A
dHC
vHC
B
Pathways enriched in dHC
Cytoskeleton
Dendritic spine
Neurogenesis
Actin-binding
Transcription regulation
Compositionally biased region:Pro-rich
Postsynaptic density
Methylation
Pathways enriched in vHC
Ion transport
Short sequence motif: Cell attachment site
Neuroactive ligand-receptor interaction
Secreted
Signal peptide
Cell adhesion
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8878
2594
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316
171
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296
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mRNA and protein with opposite expression
mRNA and protein enriched in dHC
mRNA and protein enriched in vHC

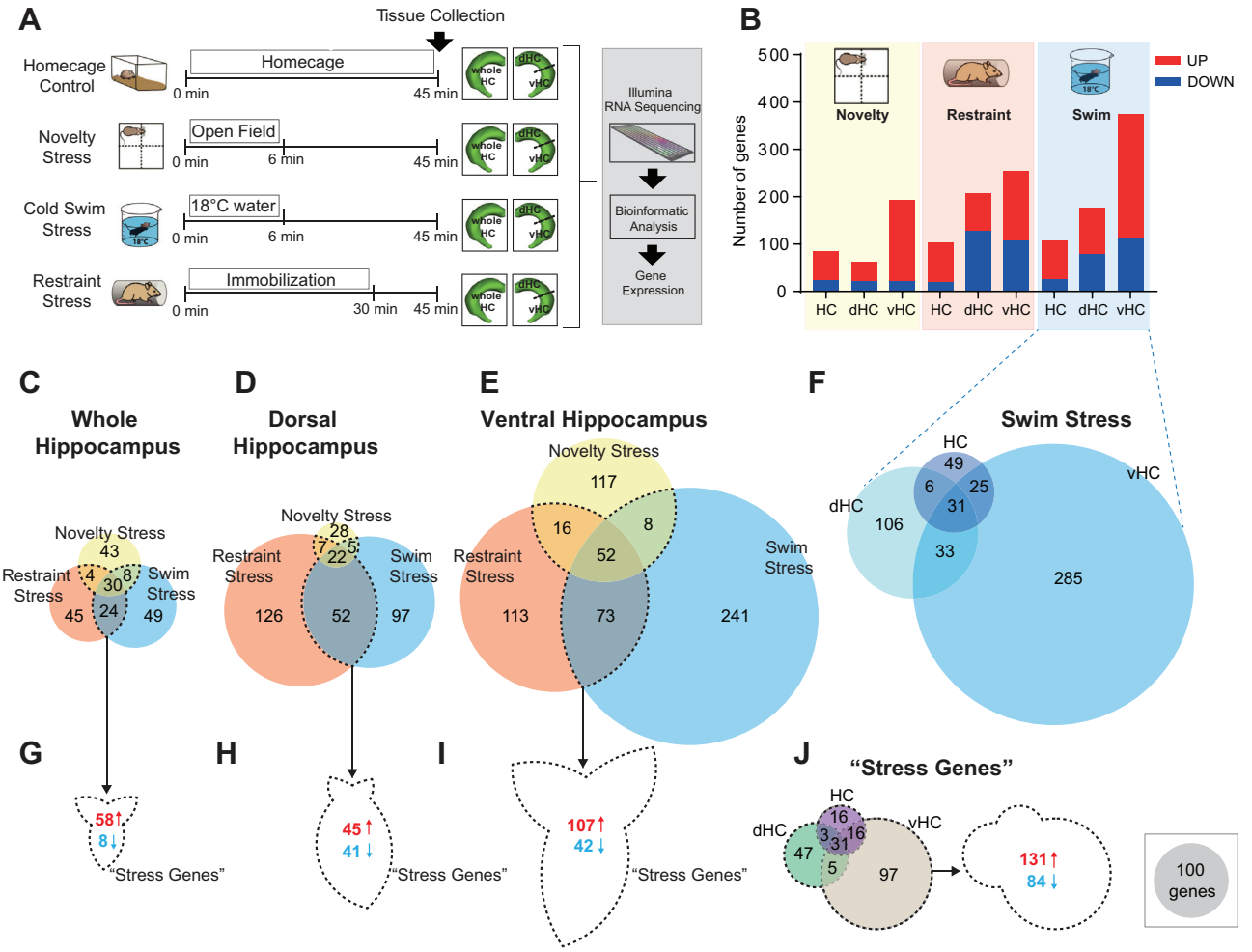

Figure 2. Transcriptomic changes in response to various acute stressors in whole hippocampus (HC), dorsal hippocampus (dHC), and ventral hippocampus (vHC). **(A)** Schematic of experimental design. **(B)** Number of messenger RNAs regulated in whole HC, dHC, and vHC after each stressor (n = 5 mice per group). **(C–E)** Venn diagrams showing overlap between individual stressors. **(F)** Venn diagram showing overlap of messenger RNAs regulated by swim stress in whole HC, dHC, and vHC. **(G–I)** Number of up- and downregulated messenger RNAs identified by more than two stressors (stress genes). **(J)** Venn diagram showing overlap between regulation of stress genes in whole HC, dHC, and vHC.

Proteomic Profiling of dHC and vHC

We then used the recently developed SWATH-MS-based proteomics approach to conduct proteome quantification [Gillet *et al.* (32)] comparing dHC with vHC (n = 6 mice/group). Across all samples, we were able to identify a total of 4564 proteins, 2414 of which we could reliably quantify. SWATH-MS measurements revealed high reproducibility with Pearson correlation coefficients of protein intensities between biological replicates of .967 to .994 in the vHC and .970 to .996 in the dHC (Figures S3 and S4 in Supplement 1, respectively). Similar to messenger RNA (mRNA) data, we observed that more than 20% of all detected proteins (513 of 2414) were differentially expressed between dHC and vHC ($p < .005$, log2 fold change $> \pm 0.3$) (Figure 1E). In total, 389 proteins were enriched in the dHC and 124 proteins were enriched in the vHC (Table S7 in Supplement 2).

For RNA sequencing and SWATH-MS, the p value distribution reveals very strong effects (Figure 1D, F). When integrating these datasets, we identified 171 genes with significant differences at both the mRNA and protein levels (Figure 1G and Table S8 in Supplement 2). The majority of these (151 genes) show a positive correlation between mRNA and protein expression (r = .56, n = 171, $p < .0001$) (Figure 1H), with 37 genes enriched in the vHC and 114 genes enriched in the dHC (Table S8 in Supplement 2). Pathway analysis revealed that mRNAs/proteins enriched in the dHC are involved in calcium signaling pathways and glutamatergic synapse function, whereas those enriched in the vHC are involved in metabolic pathways (Table S9 in Supplement 2). Among the most enriched mRNA/protein pairs, we found *Wfs1* and *Epha7*, both of which have been previously shown to be enriched in hippocampal CA1 neurons (16). This demonstrates that our proteomic analysis is able to resolve subregion-specific expression differences in heterogeneous brain dissections. To further show that SWATH-MS data can be validated independently, we used Western blotting to quantify one

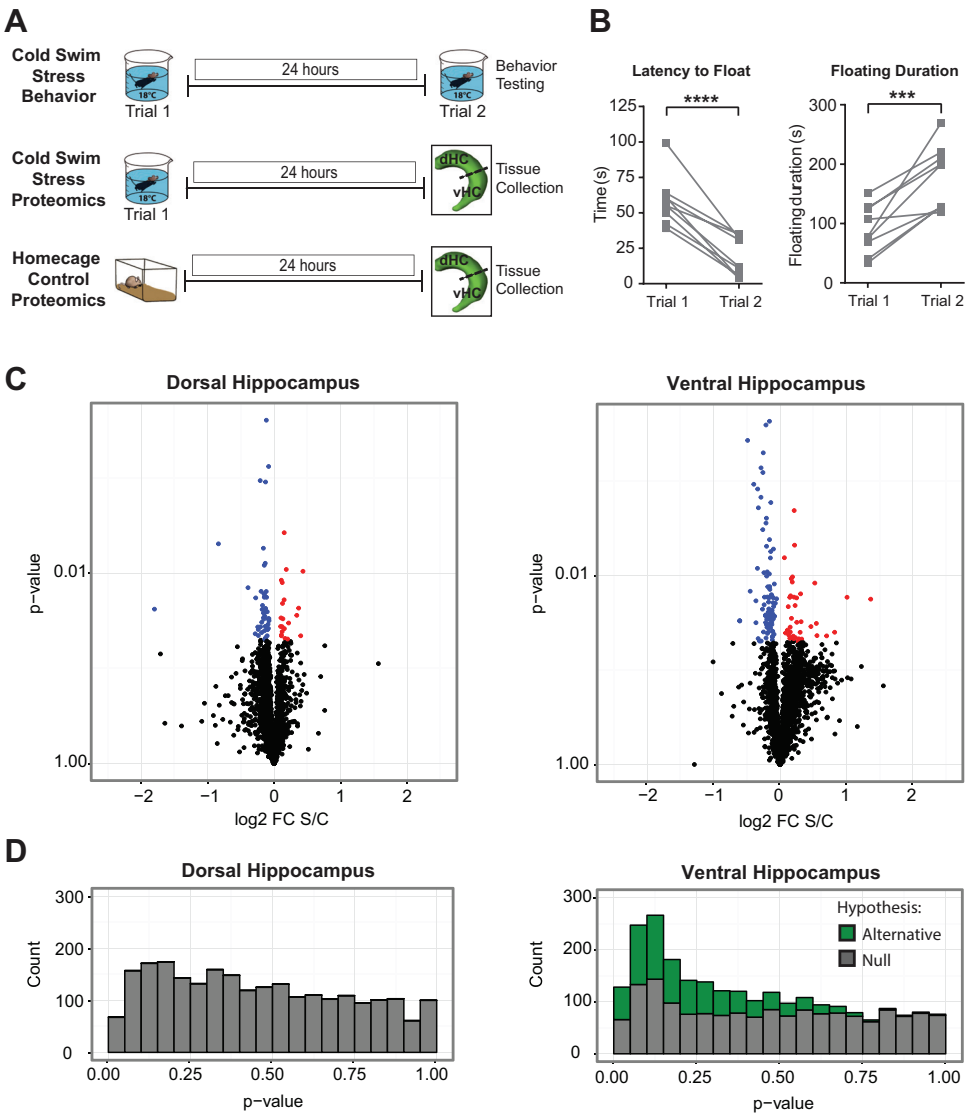

Figure 3. Long-lasting impact of stress exposure on behavior and hippocampal proteome. **(A)** Experimental design. **(B)** Behavioral assessment of the forced swim test during two test sessions 24 hours apart. In the second session, latency to float is decreased (t_8 = 5.37, p = .0007) and floating duration is increased (t_8 = 7.82, $p < .0001$, n = 9). **(C)** Proteomic analysis showing proteins significantly increased (red) or decreased (blue) 24 hours after swim stress exposure in dorsal hippocampus (dHC; left) and ventral hippocampus (vHC; right). **(D)** The p-value distribution of proteomic analyses. ***$p < .001$; ****$p < .0001$; FC, fold change; S/C, ratio of stress to control.

of the mRNA/protein pairs highly enriched in the dHC, myelin oligodendrocyte glycoprotein. We confirmed a highly significant enrichment in the dHC (t_{10} = 6.46, $p < .0001$) (Figure S5 in Supplement 1).

A small subset of genes (20 of 171) showed opposite effects on mRNA and protein levels (Table S8 in Supplement 2). These included a substantial number of receptors (e.g., *Gabra3*, *Gria4*) and ion-channel genes (e.g., *Scn9a*, *Kcnma1*, *Kcna3*, *Cacnb4*, *Cacna2d2)*. Pathway analysis revealed an enrichment for neuroactive ligand–receptor interaction and serotonergic and gamma-aminobutyric acidergic synaptic transmission (Table S9 in Supplement 2). Because serotonergic signaling was also recognized by functional cluster analysis of RNA sequencing data described above (Figure S2 in Supplement 1; Table S6 in Supplement 2), we measured monoaminergic neurotransmitter levels using high-performance liquid chromatography. Indeed, results showed higher serotonin levels in the vHC than in the dHC (t_8 = 4.28, p = .003) (Figure S6 in Supplement 1). Notably, norepinephrine and epinephrine levels, but not dopamine levels, were also higher in the vHC than in the dHC (Figure S6 in Supplement 1).

Different Stressors Induce Distinct Transcriptional Changes

Given the profound molecular differences observed between dHC and vHC, we hypothesized that stressful stimuli would elicit different molecular responses in both structures. To capitalize on the fact that stressful experiences lead to rapid gene expression changes in the hippocampus, we used RNA sequencing to assess the impact of stress on the hippocampal transcriptome. To this end, we exposed mice to one of three commonly used stress paradigms (n = 5/group): 1) novelty stress (6 minutes in a novel environment), 2) restraint stress

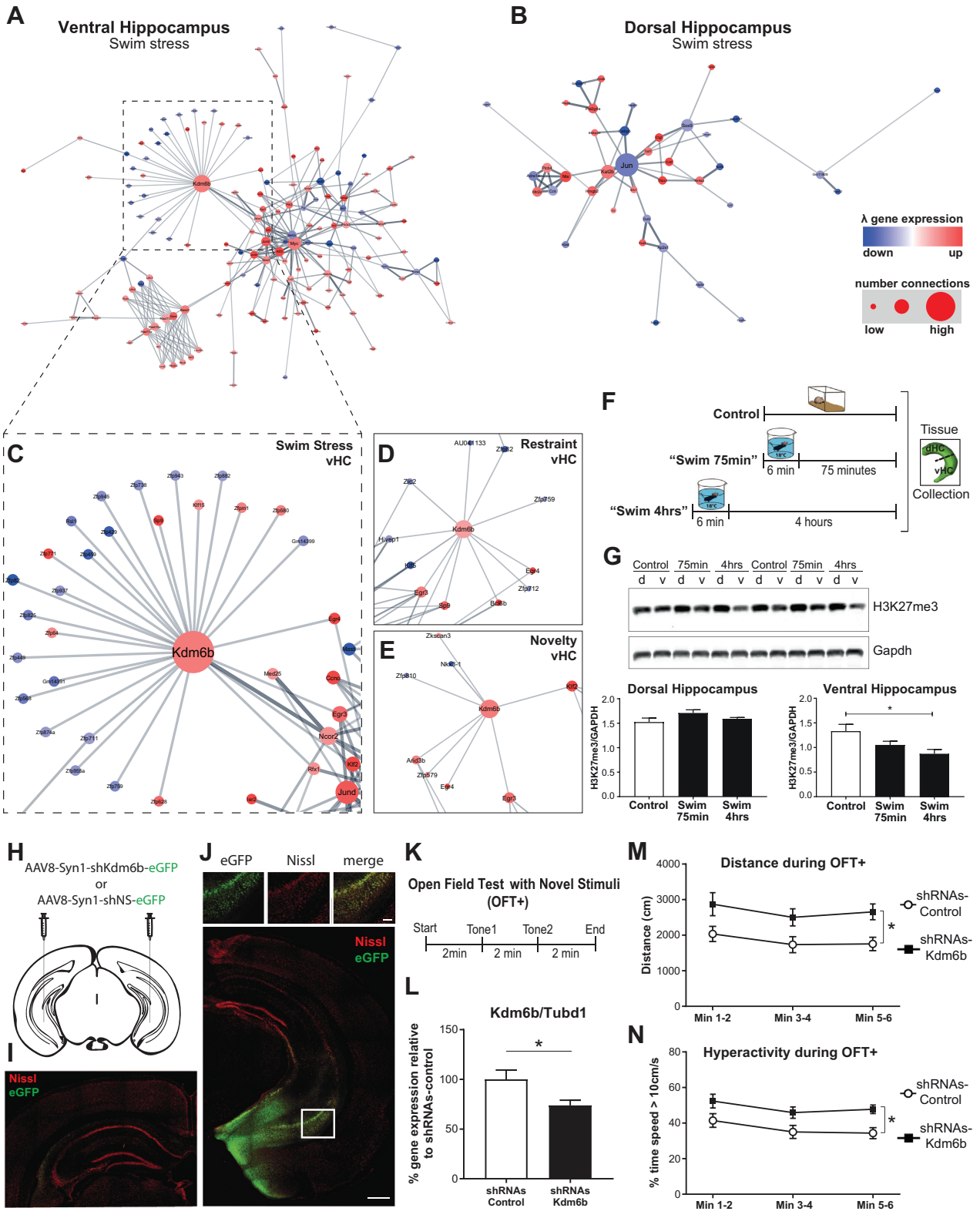

Figure 4. A stress-responsive epigenetic network in the ventral hippocampus. **(A)** Protein–protein interaction network in the ventral hippocampus (vHC) after swim stress. **(B)** Interaction network in the dorsal hippocampus (dHC) after swim stress. **(C–E)** Enlarged network cluster around *Kdm6b* in the vHC after swim stress **(C)** and after restraint stress **(D)** and novelty stress **(E)**. **(F)** Experimental design to measure H3K27me3. **(G)** Western blot analysis of H3K27me3 in dHC

(physical immobilization for 30 minutes), or 3) swim stress (forced swimming for 6 minutes in 18°C water). Mice were sacrificed for tissue collection 45 minutes after initiation of stress together with home-cage control mice (n = 5) (see Figure 2A). Hippocampi were extracted, and one hemisphere was collected as a whole (whole HC), while the other hemisphere was split into dHC and vHC. We showed that approximately 100 genes strongly responded to each stress condition in the whole HC ($p < .005$, log2 fold change $> \pm 0.3$), with the majority of genes being upregulated after stress exposure (Figure 2B). An unexpected finding was that all three stressors caused radically different gene expression profiles (for a list of all differentially regulated genes, see Table S10 in Supplement 2). In whole HC, dHC, and vHC, the number of genes uniquely regulated by each stressor was far greater than the number of overlapping/shared genes (Figure 2C–E). This prompted us to analyze the genes that changed in response to at least two stressors; here we refer to them as stress genes. We identified 215 stress genes in whole HC, dHC, and vHC (Figure 2G–J), of which 131 genes were upregulated and 84 genes were downregulated. Remarkably, all identified stress genes were regulated in the same direction (increased or decreased) consistently among stressors (novelty, restraint, and swim) within a given region (whole HC, dHC, or vHC) (Table S11 in Supplement 2). Pathway analysis revealed that the upregulated stress genes were related to transcriptional regulation, whereas the downregulated stress genes were related to cell adhesion and cell junction (Figure S7 in Supplement 1).

Strong Information Gain by Separately Analyzing dHC and vHC

When analyzing dHC and vHC separately, the number of genes identified increased dramatically (Figure 2B). While analyzing the whole HC identified 111 genes regulated by swim stress, analyzing dHC and vHC separately identified 486 genes (4.4-fold increase) (Figure 2F). Similarly, analyzing dHC and vHC separately increased the number of unique genes by 2.5-fold and 4.1-fold for novelty stress and restraint stress, respectively (Figure S8 in Supplement 1). The same held true for stress-genes. In the whole HC, we identified 66 stress genes (Figure 2G); in dHC and vHC, we identified a total of 199 stress genes, a threefold increase (Figure 2H–J). Again, vastly different sets of genes were detected in dHC and vHC (Figure 2J).

Heightened Stress Sensitivity in vHC

Our transcriptomic analyses clearly showed that the vHC is particularly responsive to stress-induced changes, given that each stressor consistently regulated more genes in the vHC than in the dHC (Figure 2B) and that the number of stress genes was twice as high in the vHC as in the dHC (Figure 2H–J). We next asked whether the heightened stress sensitivity of the vHC was also observed at the proteome level. Because swim stress induced the strongest gene expression changes overall, particularly in the vHC (Figure 2F), and because protein levels changed more slowly than mRNA levels, we decided to measure protein levels 24 hours after swim stress exposure. At this time point, stress-induced behavioral changes have been well described (35), and mice exposed to swim stress on 2 subsequent days (24 hours apart) showed a dramatic switch from active (swimming) to passive (floating) coping behaviors during the second test (Figure 3A, B). We also assessed anxiety using the open field test (36) 24 hours after swim stress exposure, and we detected an increase in anxiety as measured by increased defecation (t_{50} = 2.143, p = .037, as well as suppressed exploratory rearing (t_{50} = 2.852, p = .006) (Figure S9 in Supplement 1). We then compared proteome-wide changes 24 hours following swim stress (n = 7 mice) with nonstressed control mice (n = 6 mice) (Figure 3A). Expectedly, the observed stress-induced effects were much smaller than the profound proteomic differences between dHC and vHC (see Figure 1). Therefore, we chose a less conservative cutoff ($p < .05$) to identify significantly regulated proteins 24 hours after stress exposure. With these parameters, we identified 68 proteins changed after stress in the dHC and 128 proteins changed after stress in the vHC (Figure 3C; Tables S12 and S13 in Supplement 2). Only 3 of these proteins were significant in both dHC and vHC, which is strikingly similar to the very different transcriptional response to stress between dHC and vHC. The p-value distribution indicates a stronger overall effect in the vHC compared with the dHC (Figure 3D). In line with the stronger transcriptional changes observed in the vHC shortly after stress, these results suggest that 24 hours after stress exposure the heightened stress sensitivity of the vHC is still detectable at the proteome level.

Network Analyses Reveal an Epigenetic Cluster in vHC

To gain a better functional understanding of the molecular networks and processes differentially regulated by stress in dHC and vHC, we integrated gene expression data into protein–protein interaction networks and visualized the results using Cytoscape (see Methods and Materials for details). We observed larger interaction networks in the vHC than in the dHC (Figure 4A, B; Figures S10 and S11 in Supplement 1), and we identified one epigenetic cluster around the histone demethylase *Kdm6b* (also known as Jumonji domain containing 3 [*Jmjd3*]) in response to all stressors in the vHC (Figure 4C–E). Notably, none of these network clusters was detected in the dHC after any of the stress conditions. *Kdm6b* is a histone demethylase specific for the repressive histone mark H3K27me3 (37). Previous work has shown the ability of stress to induce epigenetic changes in the hippocampus within a few hours after stress exposure (21,22,38). Therefore, we

and vHC (n = 6–7 per group). Insets show representative images. **(H)** Cartoon showing stereotactic virus delivery to the vHC. **(I, J)** Representative microscopy images show no enhanced green fluorescent protein (eGFP) expression in neurons of the dHC **(I)** but show strong eGFP expression in the vHC **(J)**. Scale bar = 500 μm. Insets in **(J)** show that the eGFP signal is colocalized with Nissl stain and not observed in the cortex (scale bar = 100 μm). **(K)** Experimental design for the open field test with novel stimuli (OFT+). **(L)** *Kdm6b* expression is reduced in the vHC of short hairpin RNAs (shRNAs)-Kdm6b mice (n = 9–11 per group). **(M, N)** *Kdm6b* knockdown leads to increased locomotion **(M)** and hyperactivity **(N)** in the OFT+ (n = 10–11 per group). Data are presented as mean ± SEM. d, dorsal; Syn1, synapsin 1; v, ventral.

used Western blotting to quantify the level of H3K27me3 at baseline, 75 minutes, and after 4 hours following swim stress exposure (Figure 4F). Two-way analysis of variance revealed a significant interaction between brain region and stress exposure ($F_{2,30}$ = 5.33, p = .010). Follow-up analyses showed no effect of stress exposure on the dHC ($F_{2,15}$ = 2.14, p = .153) but showed a decrease in H3K27me3 specifically in the vHC 4 hours after stress initiation ($F_{2,15}$ = 4.95, p = .022) (Figure 4G). To test whether knockdown of *Kdm6b* in the vHC in vivo could have an impact on behavior, we developed an adeno-associated virus containing four short hairpin RNAs (shRNAs) against *Kdm6b* under the synapsin promoter (AAV8-hSyn1-shKdm6b-eGFP) (see Figure S12 in Supplement 1). We injected this virus (shRNAs-Kdm6b), or a control vector containing nonsilencing shRNAs (shRNAs-control), stereotactically into the vHC based on previously established coordinates (Figure 4H). Four weeks after surgery, virus expression was confirmed in the vHC (Figure 4J), restricted to neurons (Figure 4J, insets), with no expression in the dHC (Figure 4I, J). *Kdm6b* mRNA levels in whole-tissue lysates of the vHC were decreased in shRNAs-Kdm6b mice relative to control mice (t_{18} = 2.56, p = .020) (Figure 4L). However, starting 10 to 14 days after virus delivery, overt changes in health and behavior became evident in shRNAs-Kdm6b mice but not in shRNAs-control mice. We observed full motor seizures in 4 of 26 injected mice, and these mice were excluded from the experiment. In 15 of the remaining shRNAs-Kdm6b mice, we noticed marked hyperactivity during weekly routine checks and cage changing. Three weeks after surgery, we tested motor activity in the open field, yet locomotion was similar between groups and was increased in shRNAs-Kdm6b mice only during the first few minutes of the test (see Figure S13 in Supplement 1). Because shRNAs-Kdm6b mice were noticeably more agitated when moving or opening the home cage, we hypothesized that the observed changes might be related to hyperresponsiveness to novel/startling stimuli. Thus, we recorded behavior in a modified open field test adapted to prevent habituation to the test by playing brief bursts of novel sounds every 2 minutes (see Figure 4K and Supplement 1 for details). Again, mice showed increased locomotor responses during the first 2 minutes but then maintained higher motor activity throughout the duration of the test ($F_{1,19}$ = 7.50, p = .0131) (Figure 4M). We also found that shRNAs-Kdm6b mice were hyperactive, as defined by movement faster than 10 cm/sec ($F_{1,19}$ = 7.56, p = .0127) (Figure 4N). This suggests that startling stimuli prevented habituation to the environment in the shRNAs-Kdm6b group and triggered hyperactivity. *Kdm6b* in neurons of the vHC seems to be critical for maintaining normal behavioral responses to unexpected stimuli.

DISCUSSION

Different Acute Stressors Induce Distinct Transcriptional Profiles

Acutely stressful events trigger a multifaceted response involving the coordinated release of neurotransmitters, hormones, and peptides (stress mediators), which sets in motion molecular cascades that lead to long-lasting structural and functional changes across the brain (5,39). Recent work in rodents has revealed that acute stressors can enhance glutamate release, decrease spine density, induce dramatic molecular changes in hippocampus, amygdala, and prefrontal cortex, and impair cognitive and emotional behaviors for hours or even days after the initial stress exposure (40–47). It is striking that the different stressors in our study induced very different transcriptomic profiles, which might be because they trigger the same stress mediators in unique ways (5). For example, some stressors lead to stronger release of noradrenaline and serotonin (28,48,49), while others trigger higher corticosterone release (50–52). These stress mediators interact with each other; noradrenaline can further enhance the excitatory effects of glutamate signaling (53), while corticotropin-releasing hormone and corticosterone have synergistic effects on spine density and depression of synaptic responses through shared molecular pathways (54). In addition, various stressors engage different neuronal populations and circuits; for example, a single-restraint stress exposure activates different neuronal networks than exposure to a multimodal stressor of the same duration (20). Multimodal stressors arguably represent a more naturalistic scenario than single-modality stressors and induce more profound effects on cognitive performance (20,54). Finally, the duration of stressors needs to be considered when interpreting gene expression changes after stress, which are highly dynamic and often transient (28,55,56). In the current study, we decided to assess the transcriptomic profile 45 minutes after initiation of stress. However, different time points will yield different transcriptomic results, and it will be important to carefully characterize the temporal profile of stress-induced transcriptomic changes in future studies. In addition, gene expression varies dramatically along the trisynaptic circuit within the hippocampus (15). One recent study used a genetic bacTRAP approach to sequence translating mRNAs in principal neurons from the CA3 subregion of the hippocampus after stress exposure (57), an elegant approach that should be extended to other subregions and cell types in the future (58).

A Shared Transcriptomic Profile Between Different Acute Stressors

Genes regulated by at least two different stressors—here referred to as stress genes—make up only a small part of the total number of genes regulated by stress. A recent study in rats used the same three stress conditions (novelty, restraint, and swim) and showed rapid binding of both glucocorticoid and mineralocorticoid receptors (glucocorticoid and mineralocorticoid receptor heterodimers) to glucocorticoid response elements of the genes *Per1* and *Sgk1* in response to all stressors (55). We found both of these genes to be upregulated by all stressors (see "stress genes" in Table S11 in Supplement 2), suggesting that the genes identified as stress genes may constitute a core set of glucocorticoid-responsive genes.

dHC and vHC Respond Differently to Stress

Early work demonstrated that lesion of the vHC, but not of the dHC, potentiated the emergence of stress ulcers (59). Furthermore, dHC and vHC show opposite electrophysiological responses to the stress hormone corticosterone (17). One month after an acute traumatic stress experience, rats were found to be more anxious and showed increased beta-adrenergic receptor-2 binding to downstream effector proteins in the vHC but not in

the dHC (60), and the immediate early gene *Fos* was primed for activation in the vHC (47). In addition, stress induces different epigenetic changes in dHC and vHC. The activating histone mark H3S10p-K14ac is increased after acute swim stress in the dentate gyrus of the dHC, but not of the vHC, in a glucocorticoid-dependent manner (21,61,62), while H3K9me3 is increased, and H3K27me3 is reduced specifically in the dHC shortly after restraint stress (changes in the vHC were not assessed) (38,63). Targeted analysis of transcriptional changes after chronic stress in the vHC recently identified individual genes that are causally related to stress-induced depressive-like behaviors (24,25). One of these studies identified H3K27ac as an epigenetic mark specifically suppressed by chronic stress in the vHC, and linked it to reduced expression of the astroglial glutamate exchanger through the transcriptional corepressor REST (repressor element-1 silencing transcription factor) (24). H3K27ac is an activating mark mutually exclusive with the repressive mark H3K27me3, which we found to be decreased after acute stress. Therefore, acute stress appears to have the opposite effect of chronic stress, suggesting that the decrease in H3K27me3 after acute stress may be part of an adaptive response that might be overwhelmed after long-lasting chronic stress exposure and eventually lead to disease. Our attempt to knock down *Kdm6b*, the stress-activated lysine demethylase targeting H3K27me3, led to marked hyperresponsiveness to novel stimuli and induced epileptic seizures in some mice. Because acute stress induces glutamate release (2) and is known to trigger epileptic seizures (64), *Kdm6b* regulating H3K27me3 at specific loci might serve as an adaptive response keeping hippocampal excitability in check after acute stress exposure (65). This hypothesis needs to be more carefully assessed in future studies that combine acute and chronic stress and measure epigenetic changes along the trisynaptic circuit of dHC and vHC.

Proteomics, Clinical Implications, and Future Approaches

Proteins are the key regulators of biological function in health and disease; thus, there needs to be a focus on unbiased assessment of protein levels in the search of biomarkers in animal models or to study postmortem tissue in human patients (66–68). In these studies, separately analyzing dHC and vHC will boost the ability to detect biological differences in genome-wide screens. The different response of dHC and vHC to stress has important implications for many stress-related diseases with known hippocampal engagement such as depression, anxiety disorders, cognitive decline, and schizophrenia (7,69–71). For example, gene expression analyses in postmortem brain samples of patients with major depressive disorder have identified dysregulation in genes related to glutamatergic signaling (including *SNAP25, GRIA2,* and *GRIA3*) (72), and all corresponding proteins show significant expression differences between dHC and vHC in our proteomic screen at baseline (Table S7 in Supplement 2). Animal models have clearly shown that stress induces profound changes in glutamatergic signaling, particularly in the dHC (18,73,74). Notably, our proteomic data reveal that many of the glutamate receptors (GRIA1, GRIA2, GRIA3, GRIA4, GRM1, GRM2, GRM5, and GRM7) are strongly enriched in the dHC. Cluster and pathway analysis of our transcriptomic and proteomic data also reveal strong enrichment of networks related to glutamatergic signaling, dendritic spines, and postsynaptic density in the dHC (Figure 1B). This is in line with recent findings that spine density changes after stress in the dHC but not in the vHC (20). Importantly, some key changes in response to acute stress may occur on the level of protein posttranslational modifications, even at later time points after stress exposure, and thereby escape proteomic screening. For example, an increase in depolarization-evoked glutamate release 24 hours after acute stress depends on long-lasting phosphorylation of synapsin 1 without a changes in total synapsin 1 protein levels (42,75). Phosophoproteomic approaches are rapidly improving (76) and start revealing important new insights in complex in vivo systems (77). Although proteomic screens are powerful new tools with great clinical potential, they do not have the same sensitivity as transcriptomic screens owing to the fact that proteins display a higher dynamic range of expression compared with mRNA, making it more difficult to detect low-abundance proteins from whole-cell extracts (78). Separately analyzing dHC and vHC is a practical first step toward increasing information gain and can be implemented immediately in basic research and clinical studies.

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AF-S designed and conducted animal experiments, tissue extraction and processing, Western blots, surgeries, microscopy, and statistical analyses. LvZ conducted all proteomics-related work and analyses. LS conducted transcriptomic data analyses. OS designed and conducted all behavioral experiments. MP helped with animal experiments, processed tissue, and performed reverse transcription quantitative polymerase chain reactions. AR analyzed high-performance liquid chromatography (HPLC) samples. AC established and optimized HPLC analysis. BT advised on the project and designed experiments for HPLC. JB designed and supervised all experiments, analyzed data, and wrote the manuscript.

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