LSD1 modulates stress-evoked transcription of immediate early genes and emotional behavior

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Behavioral changes in response to stressful stimuli can be controlled via adaptive epigenetic changes in neuronal gene expression. Here we indicate a role for the transcriptional corepressor Lysine-Specific Demethylase 1 (LSD1) and its dominant-negative splicing isoform neuroLSD1, in the modulation of emotional behavior. In mouse hippocampus, we show that LSD1 and neuroLSD1 can interact with transcription factor serum response factor (SRF) and set the chromatin state of SRF-targeted genes early growth response 1 (*egr1*) and *c-fos*. Deletion or reduction of neuroLSD1 in mutant mice translates into decreased levels of activating histone marks at *egr1* and *c-fos* promoters, dampening their psychosocial stress-induced transcription and resulting in low anxiety-like behavior. Administration of suberoylanilide hydroxamine to neuroLSD1^{KO} mice reactivates *egr1* and *c-fos* transcription and restores the behavioral phenotype. These findings indicate that LSD1 is a molecular transducer of stressful stimuli as well as a stress-response modifier. Indeed, LSD1 expression itself is increased acutely at both the transcriptional and splicing levels by psychosocial stress, suggesting that LSD1 is involved in the adaptive response to stress.

epigenetics | stress | immediate early genes | LSD1 | SRF

Dynamic changes in neuronal chromatin through histone posttranslational modifications affect complex functions such as learning, memory, and emotional behavior (1). Seminal studies have shown that mice experiencing different forms of stress, including psychosocial stress, promote stress-related plasticity through epigenetic changes at specific genes, including brain-derived neurotrophic factor (*BDNF*) and immediate early genes (*IEG*s) (2–4). These modifications induce contrasting structural and functional changes in the hippocampus and the amygdala (5), brain areas responsible for the expression of anxiety-like behavior (5–8). A decrease in neural activity in the hippocampus caused by the loss of dendritic arbors and spines is associated with posttraumatic stress disorder and recurrent depressive illness (5). Therefore, an important challenge for molecular psychiatry is a better understanding of the epigenetic regulation of plasticity gene transcription in response to stress (9).

Lysine-Specific Demethylase 1 (LSD1) also known as lysine demethylase 1A (KDM1A) is an epigenetic transcriptional corepressor, tightly associated to Corepressor of REST (CoREST) and histone deacetylase 2 (HDAC2). It removes methyl groups from mono- and di-methylated lysine 4 of histone H3 (H3K4), erasing a histone mark of active transcription (10). In mammals, neurospecific splicing of microexon E8a generates the dominant-negative splicing isoform of LSD1 (neuroLSD1), which is required for the acquisition of proper neurite morphology inherent in neuronal maturation (11). Although conventional LSD1 acts as a constitutive repressor through its H3K4 demethylase activity, neuroLSD1 is unable to repress transcription (11, 12). It has been shown recently that neuroLSD1 lacks dimethylated H3K4 (H3K4me2) demethylase activity, confirming its role as a dominant-negative LSD1 isoform (13). The neuroLSD1-containing complex also can act as transcriptional activator by acquiring H3K9me2 and H4K20 demethylase activity (13, 14). In this context, the finding that LSD1 and neuroLSD1 share a subset of target genes (12–14) suggests that modulation of exon E8a splicing may represent a mechanism for fine-tuning the transcription of selected targets. The generation of a mouse model lacking microexon E8a (neuroLSD1^{KO} mice) allowed us to characterize neuroLSD1 as an in vivo modulator of hippocampal excitability. We reported that shifts in the LSD1/neuroLSD1 ratio in response to activity contribute dynamically to the control of neuronal excitability by participating in the transcriptional mechanism of homeostatic adaptation (15). In this work we implicate LSD1 and neuroLSD1 in shaping emotional behavior as fine tuners of psychosocial stress-evoked transcription of *IEG*s. We found that the complete lack (neuroLSD1^{KO}) or reduction (neuroLSD1 heterozygosity, hereafter neuroLSD1^{HET}) of neuroLSD1 in the mouse brain causes low anxiety-like behavior. At the molecular level, knocking out neuroLSD1 results in H3K4 hypomethylation and H3 hypoacetylation of early growth response 1 (*egr1*) and *c-fos* promoters, decreasing their stress-induced transcription. This impairment hinders the acquisition of stress-related plasticity relevant to anxiety behavior. Moreover, in wild-type animals, LSD1 transcription and alternative splicing are directly modulated by psychosocial stress, indicating that LSD1 and neuroLSD1 not only participate in the transduction of stressful stimuli but also represent stress-response modifiers.

Significance

In mammals, different forms of stress, including psychosocial stress, can affect several aspects of health, fostering mood and anxiety disorders in humans. However, a lack of knowledge about the mechanisms underlying the brain physiology of the stress response hinders the development of new therapeutic strategies. We describe the role of the epigenetic enzyme Lysine-Specific Demethylase 1 (LSD1) in the transduction pathway that translates social stress into an altered transcriptional physiology of plasticity genes in hippocampus. In particular, we show that in the brain LSD1 is finely tuned by a mammal-restricted splicing-based mechanism, and its demethylase activity consistently affects anxiety-like behavior in mice. This work addresses a fundamental mechanism, explaining a brain-related phenotype as a result of genome–environment interactions mediated by the epigenome.

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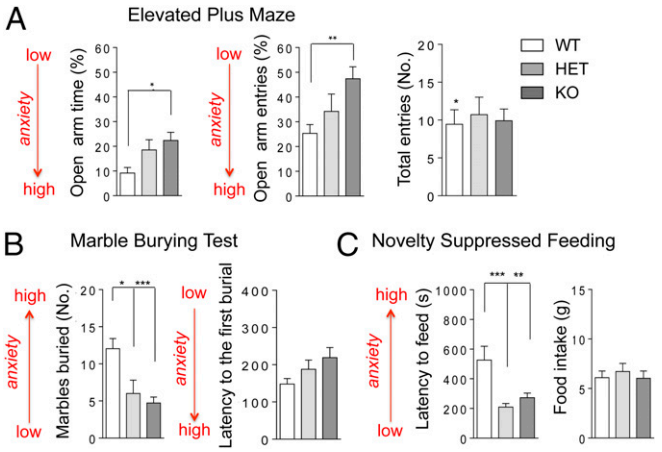

Fig. 1. NeuroLSD1-mutant mice feature a low anxiety-like phenotype. Anxiety-like behavior is evaluated by comparing neuroLSD1^{KO} and neuroLSD1^{HET} mice with wild-type littermates. The anxiety trend is shown on the *y* axis. (*A*) Time spent in the open arm time ($F_{2,27} = 4.65$, $P = 0.018$), entries into the open arm ($F_{2,27} = 6.16$, $P = 0.0063$), and total entries (which did not show any difference among genotypes) evaluated in the EPM. (*B*) Number of marbles buried ($F_{2,27} = 10.06$, $P = 0.0002$) and latency to the first burial ($F_{2,27} = 2.21$, $P = 0.12$) evaluated in the MBT. (*C*) Latency to feeding ($F_{2,27} = 9.95$, $P = 0.0003$) and total amount of food intake evaluated during the NSF test (n = 8–10 mice per genotype). Data are presented as mean ± SEM; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, one-way ANOVA, Tukey post hoc test.

Results

NeuroLSD1-Mutant Mice Display a Low-Anxiety Phenotype. To test the effect of neuroLSD1 ablation on anxiety-like behavior, we tested neuroLSD1^{KO} and heterozygous mice (Fig. S1) (15) using three classic approach–avoidance anxiety paradigms (Fig. 1). In the elevated plus maze (EPM), we observed that neuroLSD1^{KO} mice spent more time in and made more entries into the open arms than their wild-type littermates (Fig. 1*A*). A trend for increased time and number of entries in the open arms also was observed in neuroLSD1^{HET} mice. The total number of entries into each arm of the maze was comparable in all genotypes, and no differences in motor function were observed (Fig. S2). In the marble-burying test (MBT), post hoc analysis indicated a significant decrease in the number of buried marbles in both neuroLSD1^{KO} and heterozygous mice compared with wild-type littermates (Fig. 1*B*). Both genotypes showed a trend to increased latency to the first burial (Fig. 1*B*). In the novelty-suppressed feeding (NSF) test, both neuroLSD1^{KO} and neuroLSD1^{HET} mice exhibited significantly decreased latency to feed in the center of the arena compared with wild-type littermates. No difference was found in the food intake among genotypes (Fig. 1*C*). Taken together, the results of behavioral tests consistently show that complete lack (neuroLSD1^{KO} mice) or reduction (neuroLSD1^{HET} mice) of neuroLSD1 expression affects emotional behavior, resulting in a low-anxiety phenotype.

LSD1 and NeuroLSD1 Are Modulated by Stress and Regulate Stress-Evoked Transcription of *IEGs*. To shed light on the molecular mechanism whereby reduction of neuroLSD1 gives rise to the low anxiety-like phenotype, we investigated the role of LSD1 in transducing stressful stimuli into a transcriptional response in the hippocampus. We tested whether stress could modify LSD1 expression and the LSD1/neuroLSD1 splicing ratio in the hippocampus. To address this point, we took advantage of social defeat stress (SDS) paradigm (16), a psychosocial stress protocol that has been well validated with regard to face validity, ability to challenge the anxiety-like phenotype, and proven usefulness in unraveling cellular/molecular mechanisms of affective behaviors. We applied a single session of SDS and measured LSD1 expression and alternative splicing. In the hippocampus of wild-type stressed mice we found an increase of about 30% in total LSD1 protein levels (Fig. 2*A*) and a twofold increase in total

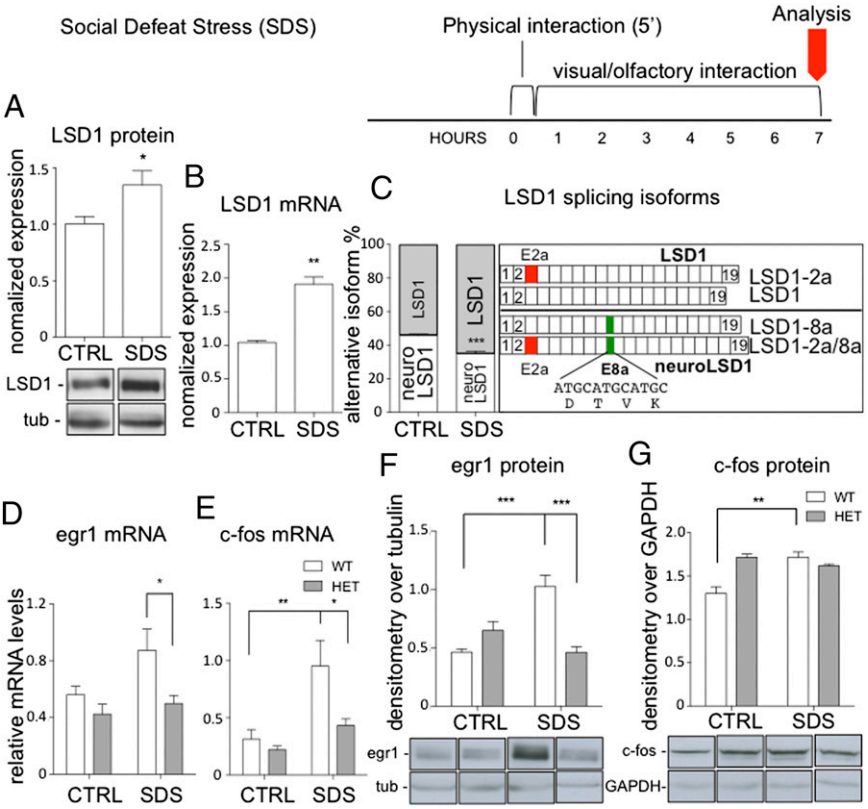

Fig. 2. Hippocampal LSD1 and neuroLSD1 levels are modified in response to psychosocial stress and modulate stress-induced transcription of *IEGs*. Mice underwent a single session of the SDS test. A schematic representation of the experimental procedure is shown at the top of the figure. (*A*–*C*) Total LSD1 protein by Western blot (*A*), LSD1 total mRNA by qPCR (*B*), and LSD1/neuroLSD1 relative percentage by rqfRT-PCR (*C*) in the hippocampi of C57BL/6N wild-type mice. A schematic representation of the four LSD1 splicing isoforms is shown on the right (n = 5 mice per group). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, Student t test. (*D*–*G*) SDS-induced transcription and protein expression of *egr1* and *c-fos* in in the hippocampi of wild-type and neuroLSD1^{HET} mice challenged with SDS. (*D* and *E*) mRNA analysis of *egr1* (treatment: $F_{1,44} = 3.561$, $P = 0.0657$; genotype: $F_{1,44} = 6.190$, $P = 0.0167$; treatment × genotype: $F_{1,44} = 1.339$, $P = 0.2535$) (*D*) and *c-fos* (treatment: $F_{1,44} = 8.865$, $P = 0.0047$; genotype: $F_{1,44} = 4.586$, $P = 0.0378$; treatment × genotype: $F_{1,44} = 2.237$, $P = 0.1419$) (*E*) (n = 10–14 mice per condition). (*F* and *G*) Western blot protein analysis of Egr1 (treatment: $F_{1,13} = 6.542$, $P = 0.0238$; genotype: $F_{1,13} = 6.612$, $P = 0.0232$; treatment × genotype: $F_{1,13} = 26,18$, $P = 0.0002$) (*F*) and C-Fos (treatment: $F_{1,13} = 8.900$, $P = 0.0175$; genotype: $F_{1,13} = 8.900$, $P = 0.0175$; treatment × genotype: $F_{1,13} = 23.69$, $P = 0.0012$) (*G*) (n = 3 or 4 mice per condition). Results are shown as mean ± SEM; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, two-way ANOVA, Bonferroni post hoc test.

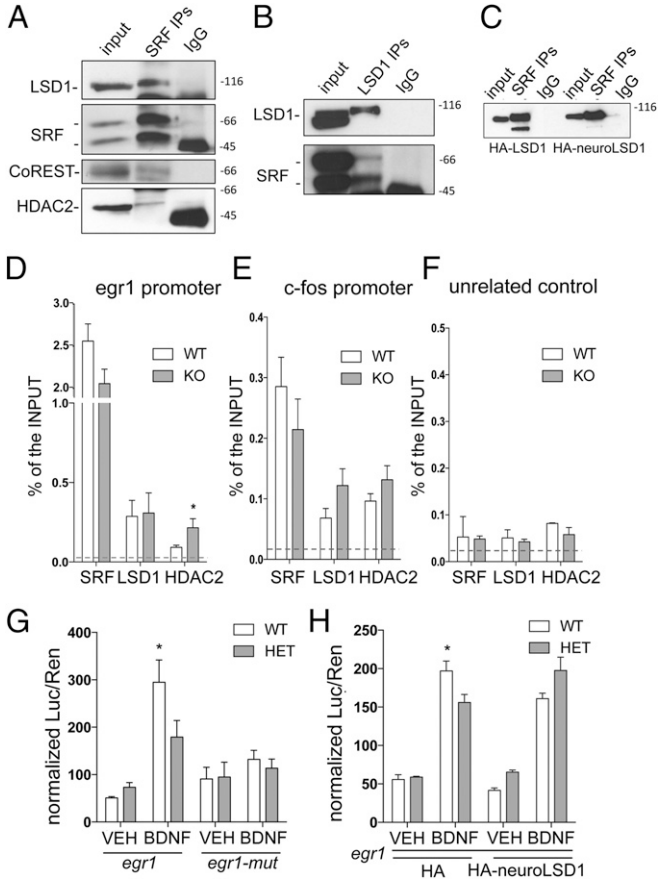

Fig. 3. LSD1, CoREST, and HDAC2 are previously unidentified SRF corepressors. (*A* and *B*) Hippocampus protein extracts immunoprecipitated with anti-SRF antibody (*A*) or with anti-LSD1 (*B*) were separated by SDS/PAGE and detected with the indicated antibodies. In addition to the 67-KDa band in *A* and *B*, the SRF antibody detected a 57-KDa band described as a neurospecific splicing isoform (28) (also see Fig. S3). (*C*) HeLa cells overexpressing HA-LSD1 or HA-neuroLSD1 immunoprecipitated with anti-SRF antibody were separated by SDS/PAGE and were immunodecorated with anti-HA. (*D–F*) qChIP experiments performed on hippocampal chromatin using anti-SRF, anti-panLSD1, and anti-HDAC2 antibodies together with IgG as mock treatment (dashed lines represent the highest mock treatment of the two genotypes) on *egr1* (*D*) and *c-fos* (*E*) promoters and an unrelated control (the *egr1* distal genomic region) (*F*). Data are shown as mean ± SEM; $*P = 0.035$, Student *t* test. (*G* and *H*) In neurons, LSD1/neuroLSD1 regulation of *IEGs*' activity-dependent transcription requires SRF binding to DNA. (*G*) Reporter assay in primary wild-type and neuroLSD1^{HET} hippocampal neurons transfected with the pGL3-*egr1*(−370) construct (treatment: $F_{1,28} = 34.74$, $P < 0.0001$; genotype: $F_{1,28} = 2.459$, $P = 0.1281$; treatment × genotype: $F_{1,28} = 5.384$, $P = 0.0278$) and the mutated version lacking the five SREs, pGL3-*egr1*(−370m) (treatment × genotype: $F_{1,20} = 0.2209$, $P = 0.6435$). (*H*) Reporter assay in primary wild-type and neuroLSD1^{HET} hippocampal neurons transfected with the pGL3-*egr1*(−370) construct and the pCGN-HA vector (treatment: $F_{1,11} = 207.2$, $P < 0.0001$; genotype: $F_{1,11} = 5.319$, $P = 0.0416$; treatment × genotype: $F_{1,11} = 7.116$, $P = 0.0219$) or pCGN-HA-neuroLSD1 (treatment × genotype: $F_{1,11} = 0.6388$, $P = 0.4411$). Reporter activity was assayed in basal conditions and after treatment with BDNF. ($n = 3$–8 per condition). Results are shown as mean ± SEM; $*P < 0.01$, two-way ANOVA, Bonferroni post hoc test.

LSD1 mRNA (Fig. 2*B*). Moreover, analysis of LSD1 splicing by relative quantity fluorescent PCR (rqf-PCR) showed that the inclusion of LSD1 exon E8a was reduced in stressed mice, resulting in the down-regulation of the neuroLSD1 isoform and a relative increase in LSD1 (Fig. 2*C*). These data indicate that in the mouse hippocampus stress induces an acute increase in LSD1 transcription as well as a change in its splicing pattern. To investigate the role of LSD1 and neuroLSD1 in the molecular pathway of stress transduction that is functionally relevant to the shaping of anxiety-like behavior, we further analyzed psychosocial stress-dependent *IEG* transcription by comparing the transactivation of *egr-1* and *c-fos* in neuroLSD1^{HET} mice and wild-type littermates. The expression of these two plasticity genes is known to mediate the translation of specific stimuli to generate inherent neuronal plasticity (4). Upon SDS, we found that Egr1 and C-Fos mRNA were transactivated and protein levels increased only in wild-type hippocampi; in neuroLSD1^{HET} animals these *IEG*s did not respond to stress (Fig. 2 *D–G*). These data indicate that a proper amount of neuroLSD1 is necessary to induce *IEG* transcription efficiently in response to psychosocial stress and thus suggest that LSD1 and neuroLSD1 have a role as stress-response transducers.

LSD1 Is a Serum Response Factor Corepressor of *IEG*s. Transcription of *egr1* and *c-fos* has been reported to be induced by SDS in mouse hippocampus (17) and to be related to the behavioral outcome of SDS in terms of stress susceptibility (4). Serum response factor (SRF), together with CREB, is an important transactivator of *egr1*, *c-fos*, and other *IEG*s (18, 19). We tested both SRF and cAMP response element-binding protein (CREB) for their ability to interact with LSD1. Using anti-SRF antibody, we coimmunoprecipitated LSD1 and the molecular partners HDAC2 and CoREST from mouse hippocampus protein extracts under resting conditions (Fig. 3*A*). Vice versa, using anti-panLSD1 antibody, we coimmunoprecipitated SRF (Fig. 3*B*). Analysis of the anti-SRF immunoprecipitates from HeLa cells stably expressing HA-LSD1 or HA-neuroLSD1 showed that both isoforms associate with endogenous SRF protein (Fig. 3*C*). Under the same conditions, we could not demonstrate molecular interaction between LSD1 and CREB (Fig. S4), suggesting that LSD1 modulation of *IEG*s could be mediated by SRF. Then we analyzed LSD1, HDAC2, and SRF chromatin occupancy at the *egr1* and *c-fos* promoters in wild-type and neuroLSD1^{KO} mice. Both promoters are known to contain serum-responsive elements (SREs) (20). In particular, five SREs cluster within the *egr1* promoter region, whereas a single SRE maps at the *c-fos* promoter. As shown in Fig. 3 *D* and *E*, quantitative ChIP (qChIP) revealed significant enrichment of LSD1 and HDAC2 together with SRF at *egr1* and *c-fos* proximal promoter regions compared with preimmune IgG in both wild-type and KO mouse hippocampi. We did not observe differences between wild-type and neuroLSD1^{KO} mice in terms of SRF and LSD1 occupancy at each promoter, but we measured a significant increase in HDAC2 enrichment at the level of *egr1* in neuroLSD1^{KO} mice. SRF is constitutively bound to its targets (21). Consistently, we did not observe psychosocial stress-induced modification of the association of SRF with the *egr1* and *c-fos* promoters (Fig. S5).

To determine the role of SRF in LSD1/neuroLSD1 modulation of activity-dependent *IEG* transcription (Fig. 2 *D* and *E*), we investigated whether SRF binding to its target sequences was required for LSD1 and neuroLSD1 to regulate *IEG* transcription. To this aim, we analyzed the effect of reduced levels of neuroLSD1 on BDNF-induced transactivation of a wild-type *egr1* reporter gene (22, 23) or a mutated version lacking the five SRE, using previously described pGL3-*egr1*(−370) and pGL3-*egr1*(−370m), respectively (20). The two reporter plasmids were transfected into hippocampal neurons derived from either wild-type or neuroLSD1^{HET} mice. The *egr1* reporter gene was activated by BDNF when transfected in wild-type neurons, but transactivation was less efficient in heterozygous neurons. Remarkably, a clear modulatory effect played by the LSD1/neuroLSD1 ratio on reporter transactivation can be evidenced only when SRF binds to SRE. Indeed, BDNF-mediated transactivation did not occur in either of the two cellular contexts when the *egr1* reporter gene carried the SRE mutation pGL3-*egr1*(−370m) (Fig. 3*G*). Importantly, BDNF-mediated transactivation of the wild-type *egr1* reporter gene was recovered in neurons derived from heterozygous mice when HA-neuroLSD1 was exogenously overexpressed (Fig. 3*H*). These data support the importance of the correct amount of neuroLSD1, in association with SRF, in eliciting

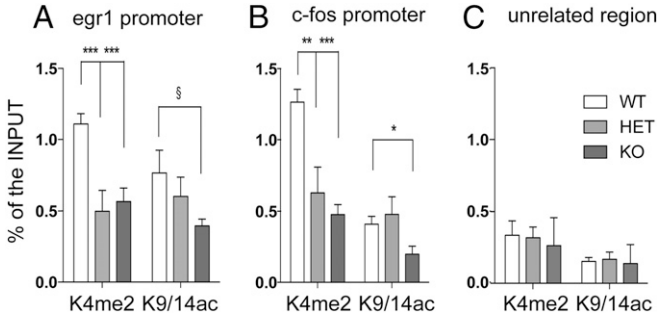

Fig. 4. NeuroLSD1^{KO} and neuroLSD1^{HET} hippocampi display reduced levels of H3K4 dimethylation and H3K9/K14 acetylation at the *egr1* and *c-fos* promoters. Hippocampal chromatin from neuroLSD1^{KO} or neuroLSD1^{HET} mice or wild-type littermates was immunoprecipitated with anti-H3K4me2 and anti-H3K9/K14ac antibodies. Enrichments are shown as percentage of input. Shown is qPCR analysis at the level of *egr1* (anti-H3K4me2: $F_{2,23}$ = 14.07, P = 0.0001; anti-H3K9/14ac: $F_{2,15}$ = 1.667, P = 0.2220) (*A*) or *c-fos* (anti-H3K4me2: $F_{2,28}$ = 19.74, P < 0.0001; anti-H3K9/14ac: $F_{2,22}$ = 4.379, P = 0.0251) (*B*) proximal promoters and the unrelated control (*egr1* distal genomic region) (*C*) (n = 5–13 per condition). Data are shown as mean ± SEM; *P < 0.05; **P < 0.01; ***P < 0.001, one-way ANOVA, Tukey post hoc test; §P = 0.0269, Bartlett's test.

IEG transactivation in response to stimuli instrumental to the acquisition of inherent forms of neuroplasticity.

NeuroLSD1-Mutant Mice Display Altered *IEG* Chromatin Structure. SRF is comparably associated with *IEG* promoters in wild-type and neuroLSD1^{KO} mice (Fig. 3 *D* and *E*). This finding suggests that, rather than being caused by differential SRF association with DNA, the impaired psychosocial stress-induced *IEG* transcription observed in neuroLSD1-mutant mice could result from altered chromatin structure at *IEG* promoters. We analyzed chromatin structure in wild-type and neuroLSD1-mutant mice by assessing their level of H3K4 methylation and H3 acetylation (H3K9/K14ac), histone marks erased by LSD1 and HDAC2. We applied qChIP to whole mouse hippocampus and found that in both neuroLSD1^{KO} and neuroLSD1^{HET} mice H3K4me2 and H3K9/K14ac were decreased at the *egr1* and *c-fos* proximal promoter regions, including SRE, compared with wild-type littermates (Fig. 4 *A* and *B*). No differences were observed among genotypes at an unrelated control region devoid of SRE (Fig. 4*C*). This specific chromatin compaction primed by LSD1-induced H3K4 hypomethylation, but also based on H3 hypoacetylation, produces minor modifications of *IEGs*' basal transcription (CTRL in Fig. 2 *D* and *E*) but may account for the defective transactivation of *egr1* and *c-fos* measured in neuroLSD1^{HET} mice in response to psychosocial stress (SDS in Fig. 2 *D* and *E*).

The Low Anxiety-Like Phenotype of NeuroLSD1-Mutant Mice Is Related to Decreased Neuroplasticity. NeuroLSD1 genetic ablation affects the psychosocial stress-dependent transcription of *egr1* and *c-fos* through direct LSD1-mediated chromatin compaction, suggesting reduced neuroplasticity. We reasoned that if the low-anxiety behavior of neuroLSD1-mutant mice is related to this peculiar molecular phenotype, reactivation of *IEG* transcription to reinstate neuroplasticity by systemic HDAC inhibition (24, 25) should restore normal anxiety. We systemically treated mice with a clinically approved HDAC inhibitor (HDACi), suberoylanilide hydroxamic acid (SAHA), 25 mg/kg i.p. for 10 d Fig. S6), to overcome *IEGs*' repressive chromatin structure observed in

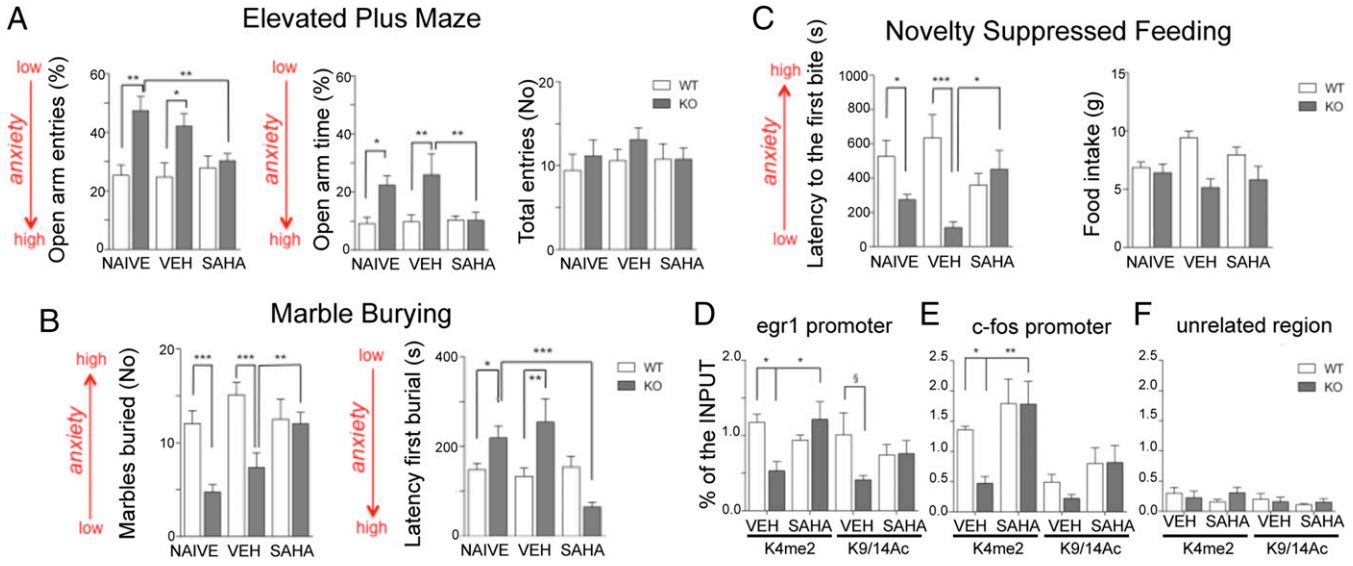

Fig. 5. Treatment with SAHA normalizes the anxiety-like phenotype of neuroLSD1 mutants. (*A–C*) Anxiety-like behavior in neuroLSD1^{KO} mice compared with wild-type mice treated with SAHA or vehicle (VEH). The anxiety trend is shown. (*A*) Entries into the open arm (treatment: $F_{2,54}$ = 1.847, P = 0.1676; genotype: $F_{1,54}$ = 19.70, P = 0.001; treatment × genotype: $F_{2,54}$ = 3.643, P = 0.032) and time spent in the open arm (treatment: $F_{2,54}$ = 5.43, P = 0.007; genotype: $F_{1,54}$ = 6.979, P = 0.0108; treatment × genotype: $F_{2,54}$ = 6.969, P = 0.002) evaluated in the EPM. (*B*) The number of buried marbles (treatment: $F_{2,54}$ = 4.436, P = 0.0165; genotype: $F_{1,54}$ = 22.35, P < 0.0001; treatment × genotype: $F_{2,54}$ = 4.566, P = 0.0147), latency to first burial (treatment: $F_{2,54}$ = 5.45, P = 0.006; genotype: $F_{1,54}$ = 2.285, P = 0.1365; treatment × genotype: $F_{2,54}$ = 7.834, P = 0.001) evaluated in the MBT. (*C*) Latency to the first bite (treatment: $F_{2,54}$ = 0.08, P = 0.92; genotype: $F_{1,54}$ = 10.29, P = 0.0023; treatment × genotype: $F_{2,54}$ = 6.26, P = 0.004) evaluated in the NSF. (n = 8–10 mice per genotype for each condition). (*D–F*) Hippocampal chromatin from neuroLSD1^{KO} or wild-type mice treated with SAHA or vehicle and immunoprecipitated by anti-H3K4me2 and anti-H3K9/K14ac antibodies. Enrichment at specific loci is shown as percentage of input. (*D*) *egr1* promoter (anti-H3K4me2, treatment: $F_{1,16}$ = 2.141, P = 0.1628; genotype: $F_{1,16}$ = 1.4405, P = 0.2468; treatment × genotype: $F_{1,16}$ = 9.080, P = 0.0082) (anti-H3K9/K14, treatment: $F_{1,14}$ = 0.0429, P = 0.8388; genotype: $F_{1,14}$ = 2.376, P = 0.1455; treatment × genotype: $F_{1,14}$ = 2.723, P = 0.1212; genotype). (*E*) *c-fos* promoter (anti-H3K4me2, treatment: $F_{1,16}$ = 11.57, P < 0.0036; genotype: $F_{1,16}$ = 3.018, P = 0.1015; treatment × genotype: $F_{1,16}$ = 2.938, P = 0.1058) (anti-H3K9/K14, treatment $F_{1,13}$ = 3.438, P < 0.0865; genotype: $F_{1,13}$ = 0,2726, P = 0.6104; treatment × genotype: $F_{1,13}$ = 0.3492, P = 0.5647). (*F*) Unrelated control (*egr1* distal genomic region) (n = 3–6 per condition). Datasets referred to as "VEH" in *D–F* are different from those in Fig. 4 in which naive mice were used. Data are shown as mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001, two way ANOVA, Bonferroni post hoc test; §P < 0.05, Student t test.

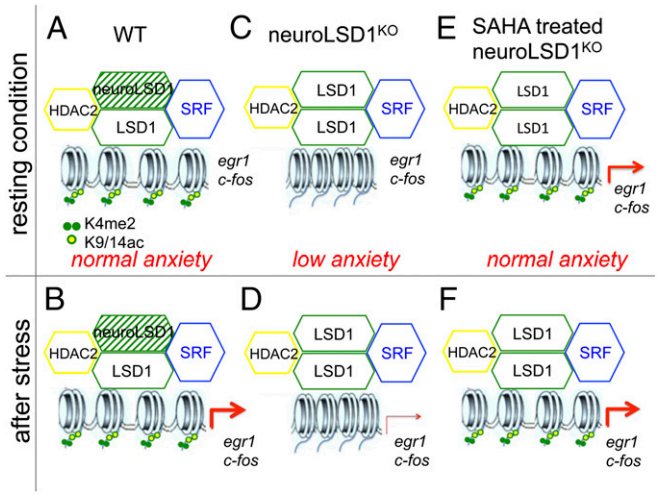

Fig. 6. Graphical model of SRF-LSD1/neuroLSD1–mediated transcriptional modulation of the *IEG*s. Given the dimeric nature of the corepressor complex, it is conceivable that, depending on the relative amounts of LSD1 and neuroLSD1, both LSD1/LSD1 homodimers and LSD1/neuroLSD1 heterodimers could be present in vivo (11). (*A*) In wild-type mice, in resting conditions, *IEG* transcription is repressed but permits activity-induced transcription. (*B*) Upon stressful stimuli, *IEG* transcription is fully activated. (*C* and *D*) In neuroLSD1-mutant mice, in resting conditions (*C*), a more condensed chromatin structure at the *IEG* proximal promoters does not permit stress-induced transcription (*D*). (*E* and *F*) Chronic treatment with the HDAC inhibitor SAHA leads to normalization of anxiety in neuroLSD1 mice.

neuroLSD1 mutants. We chose SAHA, which is able to cross the blood–brain barrier (24), for its established ability to increase hippocampal plasticity by promoting the transcription of plasticity-related genes, including *egr1* and *c-fos* (24, 26). We found that in resting conditions SAHA treatment induced sustained levels of *egr1* and *c-fos* transcription in both wild-type and neuroLSD1^{HET} mice (SAHA-treated CTRL in Fig. S7). These levels were comparable to (or higher than) those observed in wild-type mice exposed to psychosocial stress (SDS in Fig. S7), and *c-fos* levels were further increased by SDS (SAHA-treated SDS in Fig. S7). Importantly, SAHA treatment did not affect the association of SRF with its chromatin targets (Fig. S8). To analyze the effect of SAHA treatment on the anxiety-like behavior of neuroLSD1^{KO} and wild-type mice, we used the paradigms described earlier. Remarkably, SAHA significantly increased anxiety-like behavior in neuroLSD1^{KO} mice as evidenced by decreased time spent in the open arms and the decreased number of entries into the open arm as compared with vehicle-treated neuroLSD1^{KO} mice evaluated in the EPM (Fig. 5*A*). Post hoc analysis revealed that in neuroLSD1^{KO} mice SAHA restored anxiety-like behavior to wild-type levels. Similarly in the MBT the two parameters—reduced number of marbles buried and increased latency to first burial proper of neuroLSD1^{KO} mice—were restored to wild-type levels by SAHA but not by vehicle treatment (Fig. 5*B*). In the NSF test, SAHA increased the latency to feeding of neuroLSD1^{KO} mice compared with vehicle-treated neuroLSD1^{KO} mice (Fig. 5*C*). It is important to note that SAHA treatment did not exert a measurable effect on the behavior of wild-type animals, although it induced up-regulation of basal *IEG* transcription (Fig. S7). In neuroLSD1-mutant mice, poor *IEG* transcription in response to stimuli results from the LSD1/HDAC2-mediated decrease in positive histone marks at *IEG* proximal promoters (Fig. 4 *A* and *B*). Interestingly, SAHA-treated neuroLSD1^{KO} mice exhibited a significant increment in H3K9/K14ac and H3K4me2 compared with vehicle-treated mice at both the *egr1* and *c-fos* promoters (Fig. 5 *D* and *E*). The observation that the same loci were not affected by SAHA in wild-type mice suggests that the increased transcription of *IEG*s should be ascribed, at least in part, either to modifications of different histone marks at regulatory regions targeted by SRF/LSD1/HDAC2 or to changes at genomic regions not targeted by SRF, as would be expected, given the broad effect of SAHA on the overall acetylation of both histone H3 and H4 (24). We cannot exclude the possibility that SAHA-mediated increase of neuroplasticity in other anxiety-related brain structures could contribute to the normalization of anxiety in neuroLSD1 mutants.

Discussion

In this work we shed light on the molecular mechanisms underlying gene–environment interactions in the mouse brain. We propose that epigenetic enzyme LSD1 and its dominant negative isoform neuroLSD1 serve as transcriptional modulators shaping the chromatin-encoded memory of stressful experiences. In particular, we suggest that the splicing-regulated dual system LSD1/neuroLSD1 can act as a fine transduction tuner of psychosocial stress by mediating transcriptional modulation of plasticity genes. We discovered that neuroLSD1 mutants' low-anxiety behavior is related to a defective stress response, i.e., deficient psychosocial stress-induced transactivation of *egr1* and *c-fos*. Stress is known to affect the plasticity and excitability of the hippocampus (27). Together with the amygdala and prefrontal cortex, the hippocampus is part of the complex circuitry involved in mammalian trait anxiety, and its substantial role has been acknowledged (5, 7, 8, 28). We show that in the hippocampus, LSD1/neuroLSD1 repression of plasticity genes can be exerted in association with the transcription factor SRF. However, we cannot exclude the possibility that transcription factors other than SRF could contribute to recruiting LSD1/CoREST/HDAC2 to the *IEG* promoters. The association of LSD1 and neuroLSD1 with SRF is consistent with its ability to regulate the same targets either as a transcriptional activator in response to stimuli or as a repressor in basal conditions (19). SRF's repressive function has been described extensively (29); this work contributes to our understanding by characterizing the molecular basis of SRF's repressive activity. Interestingly, SRF is known to be constitutively bound to the DNA of its target genes both in resting conditions and upon stimulation (21), and its association with *IEG* promoters is not affected by psychosocial stress. Thus, the difference in the proneness of *egr1* and *c-fos* to transcription observed in wild-type versus neuroLSD1-mutant mice depends mainly on differences in the LSD1/neuroLSD1 ratio, resulting in changes in the H3K4me2 levels, in the two genotypes. When the dominant-negative neuroLSD1 isoform, lacking H3K4 demethylase activity, is low (neuroLSD1^{HET}) or absent (neuroLSD1^{KO}), the LSD1 isoform is increased or is the only one expressed. Its repressive function toward *egr1* and *c-fos* transcription is increased aberrantly, establishing a repressive chromatin state that limits the extent to which transactivation of these genes can occur in response to psychosocial stress (Fig. 6). Elevated LSD1 levels not only cause H3K4 hypomethylation but also facilitate HDAC deacetylase activity, as indicated by decreased H3K9/K14ac levels observed at the same promoters. In vitro, interdependence of LSD1 and HDAC activities has been described extensively (30), and our findings further demonstrate this functional interplay in the brain.

A central finding of this study is the discovery that in wild-type animals, in physiological conditions, LSD1 is increased and neuroLSD1 is decreased in response to stress. In heterozygous mice, the genetically produced reduction of neuroLSD1 is able per se to impact *IEG* transactivation negatively in response to stress. Thus, stress-induced neuroLSD1 down-regulation should be sufficient to buffer the stress-driven transcription of plasticity genes in a wild-type animal. This modulation could represent a possible homeostatic mechanism able to mitigate intense or prolonged stress-induced molecular responses and thereby maintain acquired plasticity at an adaptive level, although immediately after the SDS we did not observe a readout on chromatin (Fig. S9). Chronic stress results in long-lasting chromatin modifications that increase the repression of plasticity-related

genes (2–4). In particular, *egr1* has been reported to be down-regulated in mice stressed by chronic social defeat and in post-mortem brains of depressed human subjects, suggesting a pathogenic role for this down-regulation (4, 31). In this regard, it is conceivable that repeated LSD1 up- and neuroLSD1 down-regulation in response to chronic stress could induce H3K4 hypomethylation at the *egr1* promoter (Fig. S10). We have shown previously that dendrite shrinkage can be produced in cultured neurons by knocking down neuroLSD1 (11). The decrease of neuroLSD1 in the hippocampus that we observed in response to stress might represent an epigenetic mechanism instrumental in the shrinkage of dendritic arbors and loss of dendritic spines seen in stress-related psychiatric disorders (32).

SAHA treatment successfully restored *IEG* transcription and at the same time led to a phenotypical normalization of neuroLSD1 mutants' emotional behavior, suggesting that the low anxiety-like phenotype is related to reduced neuroplasticity. As expected, we also observed an SAHA-mediated increase in H3K9/K14ac and H3K4me2, histone marks specifically affected in neuroLSD1 mutants. However, because of the broad SAHA-induced chromatin remodeling (24), other effects on histone and nonhistone proteins are likely also. All our results are consistent with the idea that pharmacological targeting of LSD1 demethylase activity through specific inhibitors or by direct neurospecific splicing modulation, in combination with or as an alternative to HDACi, could represent a strategy for treating mood and anxiety disorders, including depression (33, 34). HDACi have been proposed to exert antidepressant and anxiolytic effects (34). Therefore, the SAHA-mediated restoration of anxiety in neuroLSD1-mutant mice to wild-type levels highlights an apparently counterintuitive effect of SAHA on emotional behavior. However, similar bidirectional effects of HDACi have been described in regard to memory. Indeed, SAHA and other HDACi are able to improve memory by increasing synaptic plasticity in the hippocampus (24), but they also can attenuate remote fear memories by the same molecular mechanism (25). In both these contexts, it has been suggested that HDACi act by reactivating plasticity-related genes, priming the hippocampal transcriptome to reinstate neuroplasticity (25). In conclusion we suggest that specific amounts of neuroLSD1 are necessary to couple stress exposure with its molecular response, i.e., in the expression of plasticity genes. It is tempting to speculate that neuroLSD1 could enhance mammals' ability to survive in a complex and ever-changing environment by finely tuning the molecular machinery that memorizes harmful events through stress-related plasticity.

Methods

All procedures involving animals were carried out in accordance with the European regulations (2010/63/UE) and Italian Council on Animal Care Guidelines (Legislative Decree no. 26, 2014) and were approved by the Italian Ministry of Health (no. 234/2012B and 275/2015) and by University of Milan IRB no. 27/2014. See *SI Methods* for further details of SDS procedures (16), the anxiety test (35–37), gene-expression analysis through quantitative PCR (qPCR) and rqf-PCR (11), ChIP protocols (15), primers, and antibodies.

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