ETHANOL-INDUCED NEURODEGENERATION IN NRSF/REST NEURONAL CONDITIONAL KNOCKOUT MICE

L. CAI,a,b M. BIAN,b M. LIU,a,b Z. SHENG,c H. SUO,b Z. WANG,d F. HUANGb* AND J. FEIc,d*

aLaboratory of Molecular Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China
bState Key Laboratory of Medical Neurobiology, Shanghai Medical College, Fudan University, Shanghai, China
cSchool of Life Sciences and Technology, Tongji University, Shanghai, China
dShanghai Research Center for Model Organisms, Pudong, Shanghai, China
*Graduate School, Chinese Academy of Sciences, Beijing, China

Abstract—The transcription regulator, neuron-restrictive silencer factor (NRSF), also known as repressor element-1 silencing transcription factor (REST), plays an important role in neurogenesis and various neuronal diseases such as ischaemia, epilepsy, and Huntington’s disease. In these disease processes, neuronal loss is associated with abnormal expression and/or localization of NRSF. Previous studies have demonstrated that NRSF regulates the effect of ethanol on neuronal cells in vitro, however, the role of NRSF in ethanol-induced neuronal cell death remains unclear. We generated nrsf conditional knockout mice using the Cre-loxP system to disrupt neuronal expression of nrsf and its truncated forms. At postnatal day 6, ethanol significantly increased the expression of REST4, a neuron-specific truncated form of NRSF, in the brains of wild type mice, and this effect was diminished in nrsf conditional knockout mice. The apoptotic effect of ethanol was pronounced in multiple brain regions of nrsf conditional mutant mice. These results indicate that NRSF, specifically REST4, may protect the developing brain from ethanol, and provide new evidence that NRSF can be a therapeutic target in foetal alcohol syndrome (FAS). © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: nrsf conditional knockout, ethanol, apoptosis, caspase-3, foetal alcohol syndrome.

The neuron-restrictive silencer factor (NRSF), also known as repressor element-1 (RE-1) silencing tran-
scription factor (REST), plays a key role in CNS development (Ballas et al., 2005). NRSF binds to a consensus 21-bp site, the RE1 binding site/neuron-restrictive silencer element (RE-1/NRSE) (Chong et al., 1995; Schoenherr and Anderson, 1995), and recruits histone deacetylase to depress the transcription of adjacent genes (Naruse et al., 1999). The expression of nrsf is higher in non-mature neuronal cells and lower during neurogenesis (Chong et al., 1995; Schoenherr and Anderson, 1995). The glutamate analog kainic acid increased NRSF mRNA levels in various hippocampal and cortical neurons in vivo, suggesting that NRSF has a role in neuronal activity-implied processes (Palm et al., 1998). NRSF dysfunction has been implicated in epilepsy (Spencer et al., 2006) and brain ischemia (Calderone et al., 2003; Formisano et al., 2007). Abnormal nuclear distribution of NRSF in neurons has been found in Huntington’s disease (Zuccato et al., 2003, 2007). NRSF can recruit chromatin remodeling complex and act in concert in many physiological and pathological processes. REST/NRSF-SWI/SNF complex interacts with DYRK1A to deregulate gene clusters involved in the neuronal phenotypic traits of Down syndrome (Lepagnol-Bestel et al., 2009). By recruiting Polycombs repressive complexes (PRCs) NRSF can silence target genes and actively maintain not only stem cell pluripotency but also a state of ischemic tolerance in mature neurons (Zukin, 2010). Due to alternative splicing, nrsf produces different transcripts. REST4, a neuron-specific truncated form of NRSF, is a suppressor of NRSF (Shimojo et al., 1999; Tabuchi et al., 2002). However, in epilepsy, REST4 independently regulates the expression of PPT-A (Spencer et al., 2006). The functions of REST4 in the brain are much more complex.

Foetal alcohol syndrome (FAS) is a mental and physical defect, the timing and frequency of alcohol consumption during pregnancy are the major causes for a child developing FAS (Jones and Smith, 1973). Exposure of infant mice to ethanol (EtOH), a widely accepted model to study FAS, triggers widespread apoptotic neuronal degeneration in the brain and results in a variety of neurobehavioral disturbances and even death (Olney et al., 2002a,b; Wozniak et al., 2004). Infant mice are sensitive to EtOH and such sensitivity rapidly diminishes after postnatal day 8 (P8) (Dobbing and Sands, 1979; Hamre and West, 1993). So, the effect of ethanol on the brain varies with development. It is reported that ethanol enhances in vitro NRSF binding activity in neural stem cells (Tateno et al., 2006), which indicates that NRSF is involved in the effect of ethanol on the developing brain. As a master
transcription factor in neuronal development, the function of NRSF in this process attracted our interest.

Since nrsf deficiency causes embryonic lethality before E11.5 (Chen et al., 1998), to facilitate our study of the role of NRSF in ethanol-induced neuronal degeneration, the nrsf gene was conditionally knocked out in the neurons of mice. The effects of ethanol on neuronal death were studied in mutant mice and wild type controls at postnatal day 6 (P6).

**EXPERIMENTAL PROCEDURES**

**Animals**

*Generation and characterization of NSE-Cre transgenic mice.* The pNSE-Cre vector was constructed (Fig. 1A), in which the promoter for transgene expression is the 1794 bp Ecl136II–HindIII fragment from the rat neuron-specific enolase (NSE) gene (Fors-Petter et al., 1990). Purified and linearized DNA was microinjected into the pronuclei of fertilized mouse eggs isolated from superovulated (C57BL/6 cross with DBA) F1 hybrid mice. Transgenic founders were identified by polymerase chain reaction (PCR) and crossed with wild-type C57BL/6 mice to establish the NSE-Cre transgenic mice. Tissue-specific expression of Cre recombinase was verified with RT-PCR (reverse transcription-polymerase chain reaction). Further, NSE-Cre mice were crossed with Rosa26-LacZ reporter mice to detect the expression of lacZ in the brain.

*Generation of nrsfflox/flox mice.* The nrsf locus was targeted by insertion of a loxP site in intron 1 and a pLoxpneo cassette in intron 2. Thus, the second exon of the gene containing the start codon was flanked with loxP sites and could be deleted by conditionally expressed Cre recombinase. The vector design is shown in Fig. 2A. Targeting vector DNA was electroporated into 129 mouse embryonic stem cells (ES/CJ7). Positive clones were selected with G418 and ganciclovir, and verified by PCR and sequencing. Recom-

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**Fig. 1.** Generation of NSE-Cre transgenic mice. (A) Diagram of transgene construction, prNSE: rat NSE promoter. (B) Expression of Cre recombinase in tissues of NSE-Cre transgenic mice detected by RT-PCR. (C) X-gal staining and immunohistochemical labeling of coronal brain sections of NSE-cre/ROSA26-LacZ double positive mice and control mice. (a–h) Sections of NSE-cre/ROSA26-LacZ double positive mice; (i, j) sections of Rosa26-LacZ mice as the negative control. (a–d, i) X-gal staining and immunohistochemical labeling with antibody against GFAP; (e–h, j) X-gal staining and immunohistochemical labeling with antibody against NeuN. Scale bars: (a–c, e–g, i, j): 100 μm; (d, h): 20 μm. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.
binant ES cells were injected into mouse blastocysts to produce chimeras. The chimeras were crossed with C57BL/6 mice to produce heterozygous nrsf gene floxed offspring. Homozygotes were obtained by intercrossing heterozygotes. PCR primers were as follows: 5' arm: primer 1: 5'-AATGCCATTTTCCAGGAAAGCC-3'; primer 2: 5'-TGCGACTCGGATGATACACT-3'; 3' arm: primer 3: 5'-CGCTTCTGTAGGTCTTTCTG-3'; primer 4: 5'-CCAT-GTACTGGACACCTCTACCT-3'. The positions of primers 1–4 are indicated in Fig. 2A.

Generation of NSE-Cre−nrsflox/flox mice and deletion of nrsf in neuronal cells. To delete nrsf in neuronal cells, nrsflox/flox mice were crossed with NSE-Cre transgenic mice, generating NSE-Cre−nrsflox/flox mice hereafter referred to as KO mice. Knockout of nrsf exon 2 in KO mice deleted the initiation codon of nrsf transcript gene. The deletion of the loxP-flanked fragment was confirmed by PCR and validated by sequencing (primer 5: 5'-TGTAGAACCAGCCG-TATTTGA-3'; primer 6: 5'-GGCGGCAAGACTGATACACT-3'). The positions of primers 5 and 6 are indicated in Fig. 2A. Primers used for quantitative RT-PCR analysis are listed in Table 1.

Mice were group-housed under a 12-h light/dark cycle (on between 7:00 AM and 7:00 PM) with unrestricted access to food and water. The Institutional Animal Care and Use Committee approved all animal experiments.

X-gal staining and immunohistochemistry

To examine Cre-mediated recombination in the brain, NSE-CRE mice were crossed with ROSA26-lacZ reporter mice (Soriano, 1999). Brains obtained from their progeny were processed for X-gal staining.

Tissues were collected from progeny mice anesthetized with 10% chloral hydrate and perfused intracardially with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The cell lysate was examined by Western blot analysis using mouse anti-GAPDH (MAB2166, CHEMICON, CA, USA) and rabbit anti-active caspase-3 (#9661, Cell Signal, MA, USA). Western blot results were analyzed using Quantity One 4.5.2 software (Bio-Rad, Hercules, CA, USA), and the error bar indicates the statistical differences in three analytical results.

Ethanol treatment

The infant control and mutant mice at postnatal day 6 (P6) received two s.c. injections of EtOH over a 2-h interval at a total dose of 5, 6, or 7 g/kg (Ieraci and Herrera, 2006; Olney et al., 2002a,b; Wozniak et al., 2004; Young et al., 2003). Mice receiving a dose of 5, 6, or 7 g/kg (Ieraci and Herrera, 2006; Olney et al., 2002a,b; Wozniak et al., 2004; Young et al., 2003) were anesthetized with 10% chloral hydrate and perfused intracardially with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The cell lysate was examined by Western blot analysis using mouse anti-GAPDH (MAB2166, CHEMICON, CA, USA) and rabbit anti-active caspase-3 (#9661, Cell Signal, MA, USA). Western blot analysis was performed as described previously (Cai et al., 2006).

Western blot results were analyzed using Quantity One 4.5.2 software (Bio-Rad, Hercules, CA, USA), and the error bar indicates the statistical differences in three analytical results.

Real-time PCR

Real-time PCR was performed on a Rotor-Gene 3000 instrument (Corbett Research, Sydney, Australia) using the Dynamo SYBR Green qPCR Kit (Finzymes, Espoo, Finland). For each experiment, a standard curve for each primer set was generated and used to derive the relative amounts in the samples. Melting curve analysis was carried out to confirm the specificity

For measurement of the density of cleaved caspase-3 positive cells in the hippocampus, we performed total cell counting and stereological counting under a light microscope in a double-blind fashion. Four 50 µm sections were captured under a light microscope, and positive-labelled cells were counted manually. Sections were selected every eight sections per mouse.

Fluorescence immunolabeling

For double fluorescence labeling, sections were stained with rabbit polyclonal anti-cleaved caspase-3 (Asp175) (1:500) (#9661, Cell Signal, MA, USA), rabbit polyclonal anti-NRSF (1:200) (#ab21635, Abcam), mouse monoclonal anti-neuronal nuclei (NeuN) (1:200) (RMAB377, CHEMICON, CA, USA), mouse monoclonal anti-GFAP (1:100) (MS-1376, Lab Vision, MA, USA). The sections were incubated with the primary antibodies for 48 h at 4 °C. After washing with PBS, the sections were incubated with anti-mouse IgG-FITC (1:50) (sc-2010, Santa Cruz, USA) and anti-rabbit IgG-Texas Red (1:100) (sc-2780, Santa Cruz, CA, USA) for 1 h at 37 °C. Finally, the sections were incubated with 4',6-diamidino-2-phenylindole (DAPI) at 1.5 µg/ml for 5 min at room temperature. After washing, sections were mounted on glass slides. Images were obtained using a Leica TCS SP2 confocal microscope (Leica, Wetzlar, Germany). Positive cells were verified in x-y cross-section, as well as in x-z and y-z cross-sections produced by orthogonal reconstruction from z-series.

Immunohistochemistry

Activated caspase-3 was examined using an immunohistochemical method as reported previously (Bian et al., 2008). Briefly, mice were anesthetized with 10% chloral hydrate and perfused intracardially with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brain was removed and fixed in 4% paraformaldehyde for 6 h, then immersed in 30% sucrose. Frozen 50 µm sections were prepared on a freezing microtome.
Fig. 2. Generation of nrsf conditional knockout mice. (A) Targeting strategy and vector design. A neomycin resistance (Neo) cassette flanked by loxP sequences was inserted into intron 2 for positive selection, and the thymidine kinase gene (TK) was included for negative selection. A third loxP sequence was inserted into intron 1. (B) Positive targeting events were identified by PCR with primer 1 and 2, 3 and 4, and sequencing. Primers 1 and 4 are located outside the targeting sequence and indicated by arrows in (A). Six homologous recombinant ES clones were obtained. (C) Generation of floxed mice. Primers 1 and 2 were used for offspring genotyping. WT, nrsf<sup>+/+</sup> mice; het, nrsf<sup>flox/+</sup> mice; H, nrsf<sup>flox/flox</sup> mice.
of the products between 65 and 95 °C with 0.2 °C increments. The primer sequences are shown in Table 1. A BLASTN search confirmed specificity of the chosen primer sequences for their target genes. Thermal cycling conditions were: 95 °C for 2 min, followed by 45 cycles of 94 °C for 10 s, 60 °C for 20 s, 72 °C for 20 s.

Results were analyzed using Rotor-Gene 5.0 software from Corbett Research (Sydney, Australia). Program default settings were used to define both the threshold value and baseline for analysis of the raw data. Relative expression of target genes was normalized against GAPDH. All experiments were carried out in triplicate.

Data analysis

Data were analyzed by one-way ANOVA followed by Bonferroni posthoc analysis and represented as the mean ± SEM. Statistical software was OriginPro 7.0 (OriginLab, MA, USA). \( P < 0.05 \) was considered statistically significant.

RESULTS

Generation of NSE-Cre transgenic mice and selective deletion of \( nrsf \) in the neuron of cKO mice

The Cre-loxP strategy was adapted to knock out \( nrsf \) in neuronal cells. First, NSE-Cre transgenic mice were created, in which the rat NSE promoter drove Cre recombinase expression (Fig. 1A). The transcriptional expression of Cre recombinase was limited to the brain (Fig. 1B).

To determine the activity and specificity of the NSE promoter in mouse brain, NSE-Cre transgenic mice were crossed with ROSA26-lacZ reporter mice. Cre recombinase activity was determined in offspring containing both NSE-Cre and ROSA26-lacZ transgenes. As expected, X-gal signals were obvious in the brain sections in which Cre-mediated recombination occurred. The brain sections were further immunostained with anti-NeuN or anti-GFAP antibody, and the results show that Cre recombinase activity is restricted to neurons (Fig. 1C).

The targeting vector construction for \( nrsf \) cKO is illustrated in Fig. 2A. Six recombinant ES cell clones were obtained (Fig. 2B). After microinjection of recombinant ES clones and breeding, we obtained heterozygous F1 and floxed homozygous offspring (Fig. 2C).

\( nrsf^{floxed} \) mice were crossed with NSE-Cre transgenic mice. The expected \( nrsf \) fragment lacking exon 2 was detected in the cortex, hippocampus, and remaining part of the brains of NSE-Cre \(^{+} \) \( nrsf^{floxed} \) mice, but not in \( nrsf^{floxed} \) mice (Fig. 2D).

NSE-Cre \(^{+} \) \( nrsf^{floxed} \) mice were intercrossed, and NSE-Cre \(^{-} \) \( nrsf^{floxed/floxed} \) mice (cKO) were obtained following Mendel’s law. The quantity of total \( nrsf \) mRNA in cKO mice, relative to that in \( nrsf^{floxed/floxed} \) mice (cre(−) H mice), was 82%±0.03 in the cortex, 70%±0.03 in the hippocampus, and 76%±0.05 in the remaining part of the brain (Fig. 2E). The remaining part includes the thalamencephalon, hypothalamus, and brain stem. Relative amount of REST4 mRNA in the cKO mice was 68%±0.05 in the cortex, and 63%±0.04 in the hippocampus (Fig. 2E). There is no specific change in the cerebellum (data not shown).

To confirm the knockout of NRSF in neurons of cKO mice, we did double fluorescence labeling using antibodies against NRSF and neuronal marker NeuN. The confocal imaging was conducted on the brain slices of cKO and WT mice. NRSF positive signals were only co-localized with NeuN in WT mice, but not in cKO mice (Fig. 2F).

EtOH treatment induces the expression of REST4 in wild type mice but not in cKO mice

The infant wild type mice at P6 were treated with ethanol at a dose of 5 g/kg and sacrificed at 0, 5, 10, 18, and 24 h. Ethanol increased the expression of REST4 by 2.9-fold in the cortex and 2.3-fold in the hippocampus over saline controls, while the expression of full-length NRSF did not change significantly (Fig. 3). However, in cKO mice, the effect of 5 g/kg ethanol on REST4 expression was much attenuated (Fig. 3).

Increasing apoptotic effect of ethanol in the brains of cKO mice

The apoptotic effect of ethanol on neurons was indicated by cleaved-Caspase 3. In the slices from P6 mouse brains sampled 18 h after ethanol treatment, the cleaved-Caspase 3 positive cells can be observed in the hippocampus, cortex, and thalamencephalon. In the hippocampus, with the increase of the dose of ethanol, the cleaved Caspase 3 positive cells were increased both in the cKO and control mice. However, the number of positive cells were significantly higher in the slices of...
cKO mice than that of two types of control mice, and about two-fold under the treatment of 7 g/kg EtOH (Fig. 4A, B). Increasing neuronal apoptosis was also confirmed by Western blot analysis of the cleaved Caspase 3; as the ethanol dose increased, the quantity of cleaved Caspase-3 increased in the hippocampus (Fig. 4C). Such an increase of apoptotic cells also occurred in the cortex (Fig. 4D, E) and other areas (including the thalamencephalon, hypothalamus, and brain stem) in cKO mice (data not shown). In the corpus striatum and cerebellum, there are no obvious changes (data not show). Ethanol induces apoptosis in neuronal cells but not in glial cells (Bauer-Moffett and Altman, 1977; Goodlett et al., 1990; Ikonomidou et al., 2000; Ryabinin et al., 1995; West et al., 1986). To confirm whether the increased apoptotic cells in cKO mice are restricted to neuronal cells, confocal microscopic imaging was conducted on the brain slices of ethanol-treated cKO mice. Cleaved Caspase 3 positive signals were co-localized with the neuronal marker NeuN (Fig. 5A–C), whereas the cleaved Caspase 3 positive signals were not visible in the GFAP-positive glial cells (Fig. 5D). Ethanol-induced apoptosis was restricted to neurons in the brains of cKO mice.

**DISCUSSION**

To elucidate the function of NRSF in the process of EtOH-induced neuronal death, both NSE-Cre transgenic mice and nrsf<sup>flox</sup>/H<sub>11001</sub> mice were created. Cre recombinase activity was determined in the brain of offspring containing NSE-Cre and ROSA26-lacZ transgenes, and most neurons in the cortex and hippocampus expressed LacZ (Fig. 1C). In our conditional nrsf knockout mouse model, exon 2 of nrsf is deleted specifically by neuronal expressed Cre recombinase. Since exon 2 is the common region in all isoforms reported in the literatures, all the isoforms of NRSF could not be produced in the neurons. NRSF widely exists in all types of cells in mouse brains and REST4 exists mainly in neurons, a 20%–30% reduction in nrsf expression was detected in the brains of cKO mice, while the reduction of rest4 expression was more pronounced in cKO mice (Fig. 2E). And the result of immunohistochemical staining also indicated the loss of NRSF in neurons of cKO mice (Fig. 2F). The expression of rest4, but not nrsf, is significantly up-regulated by ethanol in the brains of infant wild type mice (Fig. 3). Similarly, increased expression of rest4 was reported in the mouse model of epilepsy whilst the expression of NRSF/REST was increased to a lesser extent (Spencer et al., 2006).

Neuronal apoptosis induced by ethanol was observed in multiple regions of the mouse brain, including the cortex, hippocampus, and thalamencephalon. In comparison with NSE-Cre<sup>+</sup> nrsf<sup>+</sup> mice (loxp(<sup>−</sup>)) or NSE-Cre<sup>+</sup> nrsf<sup>flox/flox</sup> mice (cre'<sup>−</sup>H<sub>11002</sub>), ethanol treatment produced significantly more apoptotic cells in cKO mice. The identification of the apoptotic cells as neurons but not glial cells in cKO mice was confirmed by confocal
**Fig. 4.** Immunohistochemical studies and Western blot analysis of the cleaved caspase-3 after the EtOH treatment in different genotypes of P6 infant mice. Samples were collected from P6 infant mice treated with different doses of ethanol (5, 6, and 7 g/kg) or saline. Brain sections were stained with cleaved caspase-3 antibody. (A) Cleaved caspase-3 staining in the hippocampus after EtOH treatment. (a–c) From mice treated with 5 g/kg EtOH; (d–f) 6 g/kg; (g–i) 7 g/kg; (j) saline control. Mouse genotypes are indicated above. Scale bar: 20 μm. (B) Statistical analysis of the number of activated caspase-3 positive cells in the hippocampus of treated mice and control. Bars represent means ± SE from three independent experiments; n=4–7 per group. (C) Western blot analysis of cleaved Caspase-3 in the hippocampus of mice treated with different doses of ethanol. (D) Cleaved caspase-3 staining in the cortex regions (a–e) of P6 infant mice treated with saline or 7 g/kg ethanol. (a) Saline; (e) Higher magnification view of (d). Scale bar: 20 μm (a–d); 100 μm (e). (E) Western blot analysis of cleaved Caspase-3 in the cortex of mice treated with ethanol. Loxp(−): NSE-Cre− nrsf+/−; cre(−)H: nrsflox/lox; cKO: NSE-Cre− nrsflox/lox. (C, D): n=3 every lane. * P<0.05, ** P<0.01.
microscopic imaging. These results suggest that NRSF (more likely REST4) plays an important role in protecting neuronal cells from apoptosis induced by EtOH in infant mouse brains. REST4 is a suppressor of NRSF (Shimojo et al., 1999; Tabuchi et al., 2002); however, REST4 can regulate gene expression independent of NRSF (Spencer et al., 2006). In this study, the expression of selected NRSF target genes and apoptosis-related genes was determined by real-time PCR (Data not shown). We did not find significant differences in the expression of apoptosis-related gene Bax, Bak, and Bcl-xl (Jurgensmeier et al., 1998; Ruiz-Vela et al., 2005; Wei et al., 2001; Young et al., 2003) in different genotypes. Glutamate receptors, GABA-A receptors (Garthwaite et al., 1988; Ikonomidou et al., 1999, 2000), and neuronal nitric oxide synthase (nNOS) (Bonthius et al., 2002; Pantazis et al., 1998) are implicated in EtOH-induced neuronal apoptosis. The expression of these genes did not vary significantly. We also did not find significant differences in the expression of some sodium and potassium channels, which are regulated by NRSF. Therefore, the pathway for the protective function of NRSF and/or its truncated forms in EtOH-induced apoptosis in P6 mice is still under study.

There is an apparent inconsistency between our findings and several previous studies in ischemic models. Specifically, global ischemia increased the expression of REST mRNA in CA1 pyramidal rat neurons after ischemic onset and nrsf antisense RNA rescued CA1 neurons from OGD-induced death (Calderone et al., 2003; Formisano et al., 2007). The discrepancy may be explained by the following facts. First, the apoptotic insults are different so perhaps, two stimuli may induce neuronal death through different pathways. Secondly, animals of different ages were used in the experiments. Adult mice were adopted for ischemia modeling (Calderone et al., 2003; Formisano et al., 2007) and infants at P6 for ethanol treatment (Hamre and West, 1993). Thirdly, experiments with nrsf antisense RNA were acquired in vitro, whereas our experiment was conducted in vivo. These results indicate that the nrsf gene may have different functions depending on the stage of neuronal development, injurious stimuli, and cellular envi-

Fig. 5. Confocal microscopic study of EtOH-induced apoptosis in the brain. The sections were from cKO mice treated with 7 g/kg ethanol. (A–C) Cells in slices were stained with antibodies against cleaved Caspase-3 (red), NeuN (green), and DAPI (blue); (A) Cortex region, (B) Thalamencephalon region. (C) Hippocampus region. (D) Cells in slices were stained with antibodies against cleaved Caspase-3 (red), GFAP (green), and DAPI (blue). Scale bars: 10 μm (A–C); 20 μm (D).
vironment, and that NRSF homeostasis is critical for cell survival.

NRSF plays a key role in neuronal development and maturation through epigenetic machinery; altered expression of NRSF or its truncated forms may change cellular epigenetic patterns, thus producing long-term consequences. In this paper, we find that ethanol can change the expression of nrsf in the brain and apoptotic neuronal degeneration induced by ethanol was much more pronounced in multiple brain regions of nrsf conditional mutant mice. This may shed light on the sequelae of FAS in adulthood. Our nrsfrest neuronal conditional knockout mice will facilitate further research into the roles of NRSF in many neural disorders.

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