# Striatal-Enriched Protein Tyrosine Phosphatase—STEPs Toward Understanding Chronic Stress-Induced Activation of Corticotrophin Releasing Factor Neurons in the Rat Bed Nucleus of the Stria Terminalis

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**Background:** Striatal-enriched protein tyrosine phosphatase (STEP) is a brain-specific protein tyrosine phosphatase that opposes the development of synaptic strengthening and the consolidation of fear memories. In contrast, stress facilitates fear memory formation, potentially by activating corticotrophin releasing factor (CRF) neurons in the anterolateral cell group of the bed nucleus of the stria terminalis ($BNST_{ALG}$).

**Methods:** Here, using dual-immunofluorescence, single-cell reverse transcriptase polymerase chain reaction, quantitative reverse transcriptase polymerase chain reaction, Western blot, and whole-cell patch-clamp electrophysiology, we examined the expression and role of STEP in regulating synaptic plasticity in rat $BNST_{ALG}$ neurons and its modulation by stress.

**Results:** Striatal-enriched protein tyrosine phosphatase was selectively expressed in CRF neurons in the oval nucleus of the $BNST_{ALG}$. Following repeated restraint stress (RRS), animals displayed a significant increase in anxiety-like behavior, which was associated with a downregulation of STEP messenger RNA and protein expression in the $BNST_{ALG}$, as well as selectively enhancing the magnitude of long-term potentiation (LTP) induced in Type III, putative CRF neurons. To determine if the changes in STEP expression following RRS were mechanistically related to LTP facilitation, we examined the effects of intracellular application of STEP on the induction of LTP. STEP completely blocked the RRS-induced facilitation of LTP in $BNST_{ALG}$ neurons.

**Conclusions:** Hence, STEP acts to buffer CRF neurons against excessive activation, while downregulation of STEP after chronic stress may result in pathologic activation of CRF neurons in the $BNST_{ALG}$ and contribute to prolonged states of anxiety. Thus, targeted manipulations of STEP activity might represent a novel treatment strategy for stress-induced anxiety disorders.

**Key Words:** Anxiety, bed nucleus of the stria terminalis, BNST, chronic stress, corticotrophin releasing factor, CRF, STEP, striatal-enriched protein tyrosine phosphatase

Striatal-enriched protein tyrosine phosphatase (STEP) (also known as protein tyrosine phosphatase nonreceptor type 5) is a brain-specific tyrosine phosphatase that is highly expressed in regions involved in learning and memory, such as the striatum, neocortex, amygdala, and hippocampus (1,2), where it contributes to the regulation of synaptic plasticity and cognitive function, for review see (3)]. Functionally, STEP inactivates several kinases by dephosphorylating a regulatory tyrosine (Tyr) within their activation loop. Target substrates include extracellular signal-regulated kinase 1 and 2 (ERK1/2) (4), the stress-activated protein kinase p38 (4,5), the Src family tyrosine kinase Fyn (6), and proline-rich tyrosine kinase 2 (7). In addition, STEP dephosphorylates regulatory Tyr residues in subunits of *N*-methyl-D-aspartate (NMDA) (GluN2B, formerly known as NMDA receptor [NMDAR] subtype 2B) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors, promoting internalization of GluN1/GluN2B and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor complexes (3,8,9). Dephosphorylation of these proteins significantly attenuates the development of synaptic plasticity and the consolidation of memories (10–14).

Consistent with these findings, STEP blocks long-term potentiation (LTP) in amygdala slices (15), while STEP-deficient mice show facilitated LTP and amygdala-dependent fear conditioning (16). Moreover, knockdown of STEP in the dorsal hippocampus delayed physiological recovery from stress, whereas STEP overexpression enhanced resilience to stress (17). Consequently, it has been suggested that downregulation of STEP function plays a role in the etiology of stress-induced anxiety disorders, such as posttraumatic stress disorder and generalized anxiety disorder (17).

Two regions that play a critical role in regulating fear and anxiety-like behavior in response to stress stimuli are the central nucleus of the amygdala and the bed nucleus of the stria terminalis (BNST) (18). Both regions contain a high density of neurons that express the stress hormone, corticotrophin releasing factor (CRF) (19,20). Corticotrophin releasing factor neurons in the

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paraventricular nucleus (PVN) of the hypothalamus mediate the classic endocrine response to stress, while activation of CRF neurons in the BNST is thought to initiate the behavioral response to stress (18), and stress is known to modulate synaptic plasticity in the BNST (21,22). The BNST is a heterogeneous structure consisting of several distinct nuclei, which differentially regulate the stress axis (23) and anxiety-like behavior (24). Here, we have focused on the anterolateral cell group of the BNST ($BNST_{ALG}$), which contains a high density of CRF neurons (19,25,26) and is the only division showing somatodendritic immunoreactivity for STEP.

Three distinct neuronal subtypes exist in the $BNST_{ALG}$, Type I through Type III (27), of which Type III neurons are thought to be CRF neurons (26,28). However, little is known about the mechanisms that regulate synaptic plasticity in Type I through Type III neurons or the potential role of STEP in these mechanisms. In the current study, we employed a combination of immunohistochemical, molecular, electrophysiological, and behavioral techniques to examine the relative expression, distribution, and potential function of STEP in Type I through Type III neurons of the $BNST_{ALG}$ from control and stressed animals.

## Methods and Materials

### Animal Subjects

All experiments were performed in adult (45–60 days old, $n = 78$) male, Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts). For stereotaxic surgery and colchicine injections, rats were anesthetized with an intraperitoneal injection of dexdomitor (.16 mg/kg) (Pfizer Animal Health, New York, New York) and ketamine hydrochloride (48 mg/kg) (Butler-Schein Animal Health, Dublin, Ohio). All procedures were approved by the Institutional Animal Care and Use Committee of Emory University and were in compliance with National Institutes of Health guidelines. Animals were housed in same-sex groups, four animals per cage, and were maintained on a 12:12-hour light-dark cycle with ad libitum access to food and water. Animals were housed at least 1 week after arrival. Separate cohorts of control, nonstressed (NS), and repeated restraint stressed (RRS) rats were used for each experiment.

Detailed information on immunofluorescence, dual-immunofluorescence protocols, and confocal microscopy; single-cell reverse transcriptase polymerase chain reaction (RT-PCR) (scRT-PCR) from physiologically defined Type I through Type III $BNST_{ALG}$ neurons; acoustic startle response (ASR) testing; quantitative RT-PCR (qRT-PCR) of $BNST_{ALG}$ tissue samples from control and stressed rats; Western blot on the $BNST_{ALG}$ from control and stressed rats; scRT-PCR of physiologically defined Type I through Type III $BNST_{ALG}$ neurons from control and stressed rats; subcellular fractionation and NMDAR subunits expression (GluN1/GluN2B) in $BNST_{ALG}$ of control and stressed rats; and in vitro whole-cell patch-clamp electrophysiology in BNST of control and stressed rats is provided in Supplement 1.

### Repeated Restraint Stress Experiments

A standard RRS protocol was utilized to mimic the behavioral effects of chronic stress. Repeated restraint stress rats ($n = 36$)

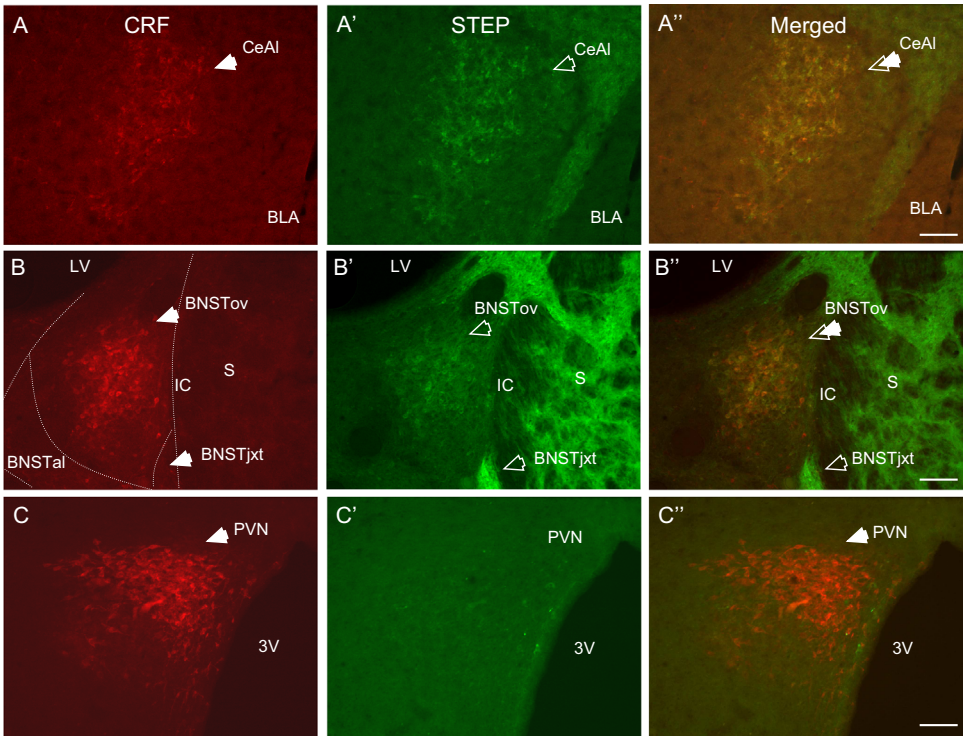


**Figure 1.** Striatal-enriched protein tyrosine phosphatase (STEP) and corticotrophin releasing factor (CRF) immunoreactivity co-localize in neurons of the lateral division of the central nucleus of the amygdala (CeAl) and anterolateral cell group of the bed nucleus of the stria terminalis $BNST_{ALG}$ but not paraventricular nucleus of the hypothalamus (PVN). **(A–A'')** CRF **(A)** (red, closed arrow) and STEP **(A')** (green, open arrow) show high somatodendritic immunoreactivity in the CeAl. Dual-label immunofluorescence revealed almost complete co-localization of STEP with CRF in neurons of the CeAl **(A'')** (double arrow). **(B–B'')** A similar pattern of co-localization was observed in neurons of the $BNST_{ALG}$ subdivision, oval nucleus of the BNST (BNSTov), but not the juxtacapsular nucleus of the BNST (BNSTjxt). **(C–C'')** STEP did not co-localize with CRF in neurons of the PVN. 10× magnification, scale bar 100 μm. BLA, basolateral nucleus of the amygdala; BNSTal, anterolateral nucleus of the BNST; IC, internal capsule; LV, lateral ventricle; S, striatum; 3V, third ventricle.