Report

Current Biology

Role of Corticotropin-Releasing Factor in Cerebellar Motor Control and Ataxia

Highlights

- Deficiency of CRF in the inferior olive induces ataxia-like motor abnormalities
- CRFergic neurons in the inferior olive project directly to the cerebellar nuclei
- CRF selectively excites the cerebellar nuclear glutamatergic projection neurons
- CRF promotes cerebellar motor coordination and rescues ataxic motor deficits

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In Brief

CRF is a critical neurotransmitter implicated in stress and anxiety. Wang et al. define a direct functional role for CRF in the olivo-cerebellar system in motor coordination via selective modulation on cerebellar nuclear glutamatergic projection neurons, and provide new insight into the etiology and treatment strategy of cerebellar ataxia.

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Role of Corticotropin-Releasing Factor in Cerebellar Motor Control and Ataxia

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SUMMARY

Cerebellar ataxia, characterized by motor incoordination, postural instability, and gait abnormality [1–3], greatly affects daily activities and quality of life. Although accumulating genetic and non-genetic etiological factors have been revealed [4–7], effective therapies for cerebellar ataxia are still lacking. Intriguingly, corticotropin-releasing factor (CRF), a peptide hormone and neurotransmitter [8, 9], is considered a putative neurotransmitter in the olivo-cerebellar system [10–14]. Notably, decreased levels of CRF in the inferior olive (IO), the sole origin of cerebellar climbing fibers, have been reported in patients with spinocerebellar degeneration or olivopontocerebellar atrophy [15, 16], yet little is known about the exact role of CRF in cerebellar motor coordination and ataxia. Here we report that deficiency of CRF in the olivo-cerebellar system induces ataxia-like motor abnormalities. CRFergic neurons in the IO project directly to the cerebellar nuclei, the ultimate integration and output node of the cerebellum, and CRF selectively excites glutamatergic projection neurons rather than GABAergic neurons in the cerebellar interpositus nucleus (IN) via two CRF receptors, CRFR1 and CRFR2, and their downstream inward rectifier K^+ channel and/or hyperpolarization-activated cyclic nucleotide-gated (HCN) channel. Furthermore, CRF promotes cerebellar motor coordination and rescues ataxic motor deficits. The findings define a previously unknown role for CRF in the olivo-cerebellar system in the control of gait, posture, and motor coordination, and provide new insight into the etiology, pathophysiology, and treatment strategy of cerebellar ataxia.

RESULTS

Downregulation of *Crf* mRNA in the Inferior Olive Induces Ataxia-like Motor Abnormalities

We found that corticotropin-releasing factor (CRF) and calbindin (a marker for inferior olive [IO] neurons [17]) were co-localized in neurons of all sub-nuclei in the IO (Figure S1), including the medial (IOM), principal (IOPr), and dorsal (IOD) nucleus (Figure 1A), which innervate the spinocerebellum. The result indicates an extensive distribution of CRFergic neurons in the IO.

To investigate the functional role of CRF in the IO, we downregulated *Crf* mRNA by *Crf*-RNAi-lentivirus (Figure 1B), which significantly decreased the level of *Crf* mRNA in the IO to 17.43% ± 0.01% ($p < 0.001$; Figure 1C). We employed footprint tests [18] to determine the effect of downregulation of *Crf* mRNA in the IO on locomotor gait. Lentivirus (LV)-*Crf*-RNAi treatment significantly shortened stride lengths ($n = 10$; left stride length: $p < 0.001$; right stride length: $p < 0.001$) and lengthened stride width ($n = 10$, $p < 0.05$) (Figure 1D). We also applied rota-rod and balance beam tests [19–21] to examine the effect of downregulation of *Crf* mRNA in the IO on motor coordination and balance. Rats treated with LV-*Crf*-RNAi displayed a remarkable decrease in the time spent on the accelerating rota-rod ($n = 10$, $p < 0.001$) (Figure 1E) and a prolonged time traversing the balance beam ($n = 11$, $p < 0.001$) (Figure 1F). These results strongly suggest that deficiency of CRF in the IO may result in cerebellar ataxia-like motor abnormalities, including gait disturbance, motor incoordination, and postural imbalance.

CRFergic Neurons in the IO Directly Project to the Cerebellar IN

Next, we microinjected the retrograde tracer Fluoro-Gold (Fluorochrome) into the cerebellar interpositus nucleus (IN) (Figure 2B), one of the two final output nodes of the spinocerebellum, for precise control of distal muscles of the limbs and digits [22–25]. We observed that the retrogradely labeled contralateral IO neurons also showed CRF immunoreactivity (Figure 2A), indicating direct CRFergic projections from the IO to the contralateral cerebellar IN. Fluoro-Gold-immunopositive fibers were also found in the inferior cerebellar peduncle (Figure 2C), through which the climbing fibers enter the cerebellum [26].

Both CRF Receptors Are Selectively Expressed and Co-localized in the Glutamatergic Neurons in the Cerebellar IN

We employed quantitative real-time RT-PCR and double immunostaining to assess the expression and distribution of two CRF receptors in the IN. The results showed that both *Crfr1* and *Crfr2* mRNAs were expressed in the IN (Figure 2D), and CRFR1 and CRFR2 (Figure 2E) were co-localized in the same IN neurons.