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Review Article

Developmental plasticity of phrenic motoneuron and diaphragm properties with the inception of inspiratory drive transmission *in utero*

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ABSTRACT

The review outlines data consistent with the hypothesis that inspiratory drive transmission that generates fetal breathing movements (FBMs) is essential for the developmental plasticity of phrenic motoneurons (PMNs) and diaphragm musculature prior to birth. A systematic examination during the perinatal period demonstrated a very marked transformation of PMN and diaphragm properties coinciding with the onset and strengthening of inspiratory drive and FBMs *in utero*. This included studies of age-dependent changes of: i) morphology, neuronal coupling, passive and electrophysiological properties of PMNs; ii) rhythmic inspiratory activity *in vitro*; iii) FBMs generated *in vivo* detected by ultrasonography; iv) contractile and end-plate potential properties of diaphragm musculature. We also propose how the hypothesis can be further evaluated with studies of perinatal hypoglossal motoneuron-tongue musculature and the use of *Dbx1* null mice that provide an experimental model lacking descending inspiratory drive transmission *in utero*.

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1. Introduction

Phrenic motoneurons (PMNs) and the diaphragmatic musculature are the major components responsible for expanding the rib cage during inspiration. Relative to many other mammalian neuromuscular systems, the PMN and diaphragm functional properties have to be in an advanced state of maturation by birth to ensure viability of the newborn. Further, the PMN–diaphragm system must be operational well before birth to generate fetal breathing movements (FBMs) *in utero*. It has been established that the expansion of the rib cage associated with FBMs *in utero* is essential for proper lung maturation (Harding and Hooper, 1996; Kitterman, 1988). Here we discuss a foundation for the hypothesis that inspiratory drive transmission that generates FBMs are also essential for the normal developmental plasticity of PMNs and diaphragm musculature properties prior to birth. Specifically, we hypothesize that the rapid maturation of PMN–diaphragm properties

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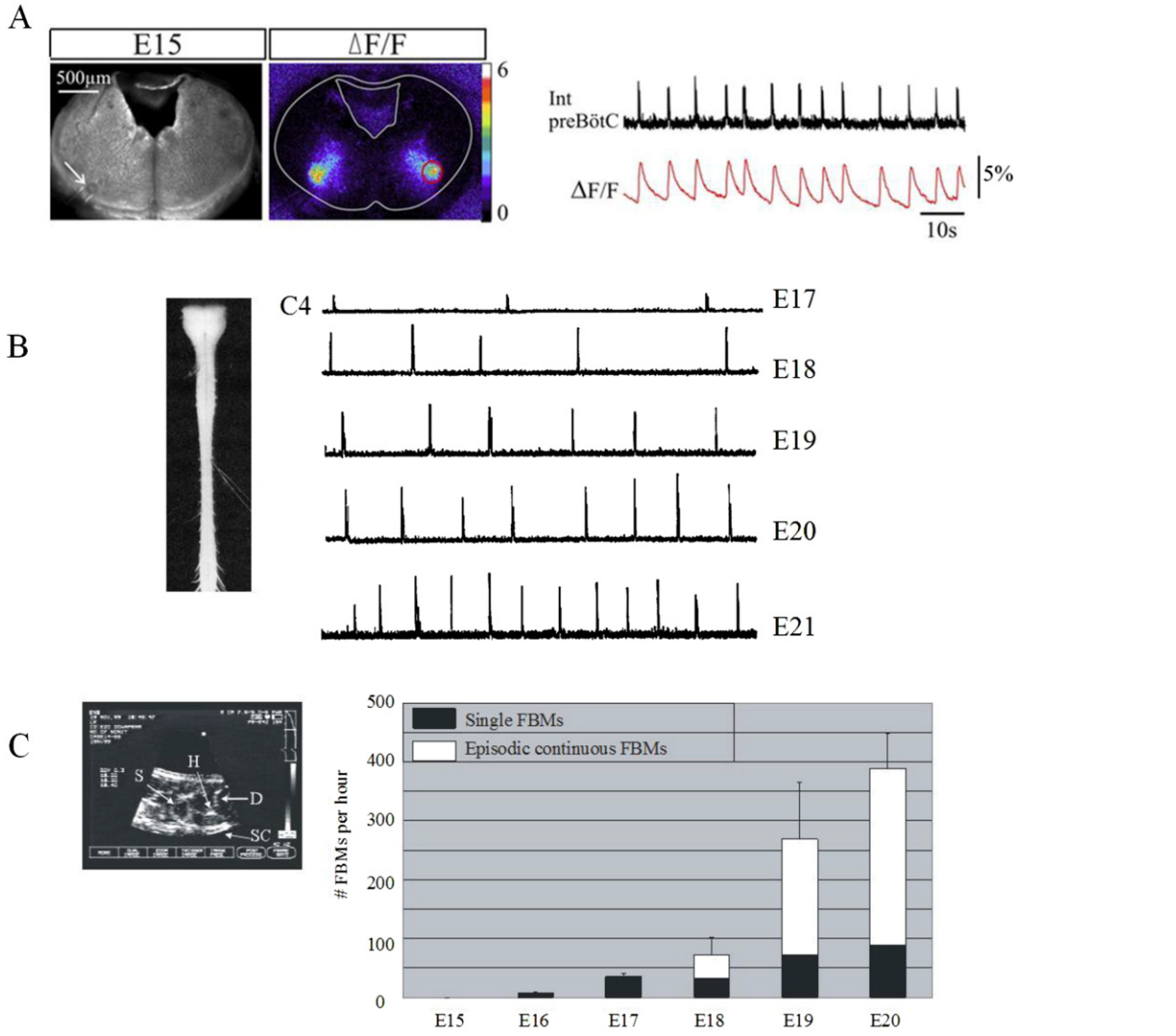

Fig. 1. *In vivo* and *in vitro* recordings of fetal respiratory activity in rodents. A) Combined calcium imaging and electrophysiological recording of respiratory rhythmic activity in E15 mouse medullary slice. Inspiratory bursts are detectable at the surface of the slice as spontaneous calcium transients illustrated as relative changes in fluorescence (red trace) and spontaneous burst of electrical activity recorded from the electrode positioned at the surface of the slice in the preBötC region. The red circle delimits the region in which calcium variations have been measured. B) Rectified and integrated suction electrode recordings of C4 ventral root activity from prenatal rat brainstem–spinal cord preparations. Rhythmic respiratory discharge commenced at E17 and the frequency and amplitude of inspiratory bursting increased in an age-dependent manner. C) Ultrasound image of fetal rat used to measure the incidence of fetal breathing movements. Right panel shows graph depicting the age-dependent increase in FBMs from E16–E20. FBMs occurred as isolated single movements or as episodes of clustered movements lasting 40–180 s. Abbreviations: H, heart; D, diaphragm; S, stomach; SC, spinal cord.
Adapted from Thoby-Brisson and Greer (2008).

after the onset of FBMs is in part due to phenotypic changes induced by activity-dependent events associated with inspiratory drive transmission. We conclude with a discussion of novel opportunities to broaden the scope of the studies and to test this hypothesis.

2. Inception of inspiratory drive transmission and fetal breathing movements

The onset of descending synaptic drive transmission from the inspiratory rhythm generating centre, the preBötzinger complex (preBötC), to PMNs and activation of the diaphragm *in utero* to generate FBMs is a major milestone in the development of the mammalian respiratory system. The timing of the inception of preBötC rhythmogenesis and inspiratory drive was determined in perinatal rodents by studies that included i) electrophysiological recordings and optical imaging from the preBötC in medullary slice *in vitro* preparations (Fig. 1A; Pagliardini et al., 2003; Thoby-Brisson et al., 2005), ii) recordings of population inspiratory discharge from the phrenic nerve of brainstem spinal cord preparations (Fig. 1B; Greer et al., 1992; Di Pasquale et al., 1996; Viemari et al., 2003) and, iii) ultrasound imaging of FBMs *in vivo* (Fig. 1C; Kobayashi et al., 2001). Rhythmic inspiratory activity was first evident within the preBötC at E17 in the rat and, fitting with the shorter gestation period of the murine model, at E15 in the fetal mouse. Recordings from rat phrenic axons demonstrated the inception of weak inspiratory motor discharge at E17 that strengthened considerably by E19. From the earliest onset of inspiratory drive transmission to PMNs *in vitro* at E17, it is blocked by antagonists to non-NMDA glutamatergic receptors (Thoby-Brisson and Greer, 2008). This is consistent with studies demonstrating that glutamate, acting largely *via* non-NMDA receptors, is the major neurotransmitter responsible for transmitting inspiratory drive to PMNs postnatally (Liu et al., 1990; Greer et al., 1991, 1992). The *in vitro* data were substantiated with *in vivo* ultrasound recordings from fetal rats that indicated an onset of weak FBMs at ~E17 that increased markedly over the subsequent 48 h. Further, anatomical studies demonstrated that by E17 in the rat, i) PMNs have migrated to their position in the medial portion of the ventral spinal cord (Allan and Greer, 1997a; Song et al., 2000), ii) presynaptic terminals on PMNs from