Brain Research Bulletin 78 (2009) 240–247

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Prefrontal afferents to the dorsal raphe nucleus in the rat

Luciano Gonçalves[a,b], Maria Inês Nogueira[a], Sara Joyce Shammah-Lagnado[b], Martin Metzger[b,*]

[a] Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, 05508-900 São Paulo, Brazil
[b] Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, 05508-900 São Paulo, Brazil

ARTICLE INFO

Article history:
Received 16 August 2008
Received in revised form
26 November 2008
Accepted 27 November 2008
Available online 25 December 2008

Keywords:
Serotonin
Prefrontal cortex
Raphe nuclei
Top-down control
Limbic
Retrograde tracing

ABSTRACT

The prefrontal cortex (PFC) receives strong inputs from monoaminergic cell groups in the brainstem and also sends projections to these nuclei. Recent evidence suggests that the PFC exerts a powerful top-down control over the dorsal raphe nucleus (DR) and that it may be involved in the actions of pharmaceutical drugs and drugs of abuse. In the light of these findings, the precise origin of prefrontal inputs to DR was presently investigated by using the cholera toxin subunit b (CTb) as retrograde tracer. All the injections placed in DR produced retrograde labeling in the medial, orbital, and lateral divisions of the PFC as well as in the medial part of the frontal polar cortex. The labeling was primarily located in layer V. Remarkably, labeling in the medial PFC was denser in its ventral part (infralimbic and ventral prelimbic cortices) than in its dorsal part (dorsal prelimbic, anterior cingulate and medial precentral cortices). After injections in the rostral or caudal DR, the largest number of labeled neurons was observed in the medial PFC, whereas after injections in the mid-rostrocaudal DR, the labeled neurons were more homogeneously distributed in the three main PFC divisions. A cluster of labeled neurons also was observed around the apex of the rostral pole of the accumbens, especially after rostral and mid-rostrocaudal DR injections. Overall, these results confirm the existence of robust prefrontal projections to DR, mainly derived from the ventral part of the medial PFC, and underscore a substantial contribution of the frontal polar cortex.

1. Introduction

The prefrontal cortex (PFC) is situated at the apex of the perception-action cycle in the brain and is critically involved in higher integrative functions such as working memory [23,49], the temporal organization of action [20], decision-making, and reversal learning [16]. Like other cortical areas, the PFC receives a dense serotonergic input mainly arising from the dorsal raphe nucleus (DR) [1,9,56,58]. However, the PFC seems unique among other cortical areas in that it receives and also sends robust direct projections to the DR [21,41,50,57; for review, see Heidbreder and Groenewegen, 27] as well as to other monoaminergic cell groups, such as the locus coeruleus [31,34] and ventral tegmental area [12,21,22]. Nowadays there is considerable evidence that specific areas in the PFC can exert a "top-down" control over monoamine systems by means of descending projections to the nuclei of origin [46].

Results from several electrophysiological, pharmacological and behavioral studies [4,14,15,26,37,42,55] strongly suggest that neuronal activity in the DR may be powerfully regulated by afferents from the PFC and that prefrontal projections to the DR may be involved in the mechanisms of action of pharmaceutical drugs and drugs of abuse [24,38] as well as in behavioral responses to stress [5,6,7].

Although the existence of a robust projection from the PFC to the DR is widely acknowledged [3,21,41,50,57], specific aspects of the anatomical organization of this pathway still need to be clarified. In particular, it is of interest to know whether the DR is primarily innervated by afferents from the ventral part (see Heidbreder and Groenewegen, [27]) or dorsal part of the medial PFC [21]. In a classic anterograde tracing study [56], it has been suggested that the rostral and caudal parts of the DR have different telencephalic outputs. The rostral DR gives rise to heavier projections to virtually all neocortical areas, and the caudal DR to the hippocampal formation. Although detailed investigations of DR afferents using sensitive retrograde tracers have been performed [21,41], it has not yet been examined whether the rostral and caudal DR receive different sets of prefrontal inputs.

In the present study, the origin of prefrontal afferents to different parts of the DR was investigated by using cholera toxin subunit b (CTb) as retrograde tracer.

2. Materials and methods

Adult male Wistar rats ($n = 42$, 160–220 g), fed *ad libitum* and kept under controlled environmental conditions (12 h light/dark cycle, light on at 7:00 a.m.; room temperature 22 °C) were used in our experiments. All the procedures were approved by the ethical committee of animal experimentation of the Institute of Biomedi-

* Corresponding author at: Depto. de Fisiologia e Biofísica, Instituto de Ciências Biomédicas I, Universidade de São Paulo, Av. Prof. Lineu Prestes 1524, 05508-900 São Paulo, SP, Brazil. Tel.: +55 11 3091 8044; fax: +55 11 3091 7285.
E-mail address: metzger@icb.usp.br (M. Metzger).

0361-9230/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2008.11.012

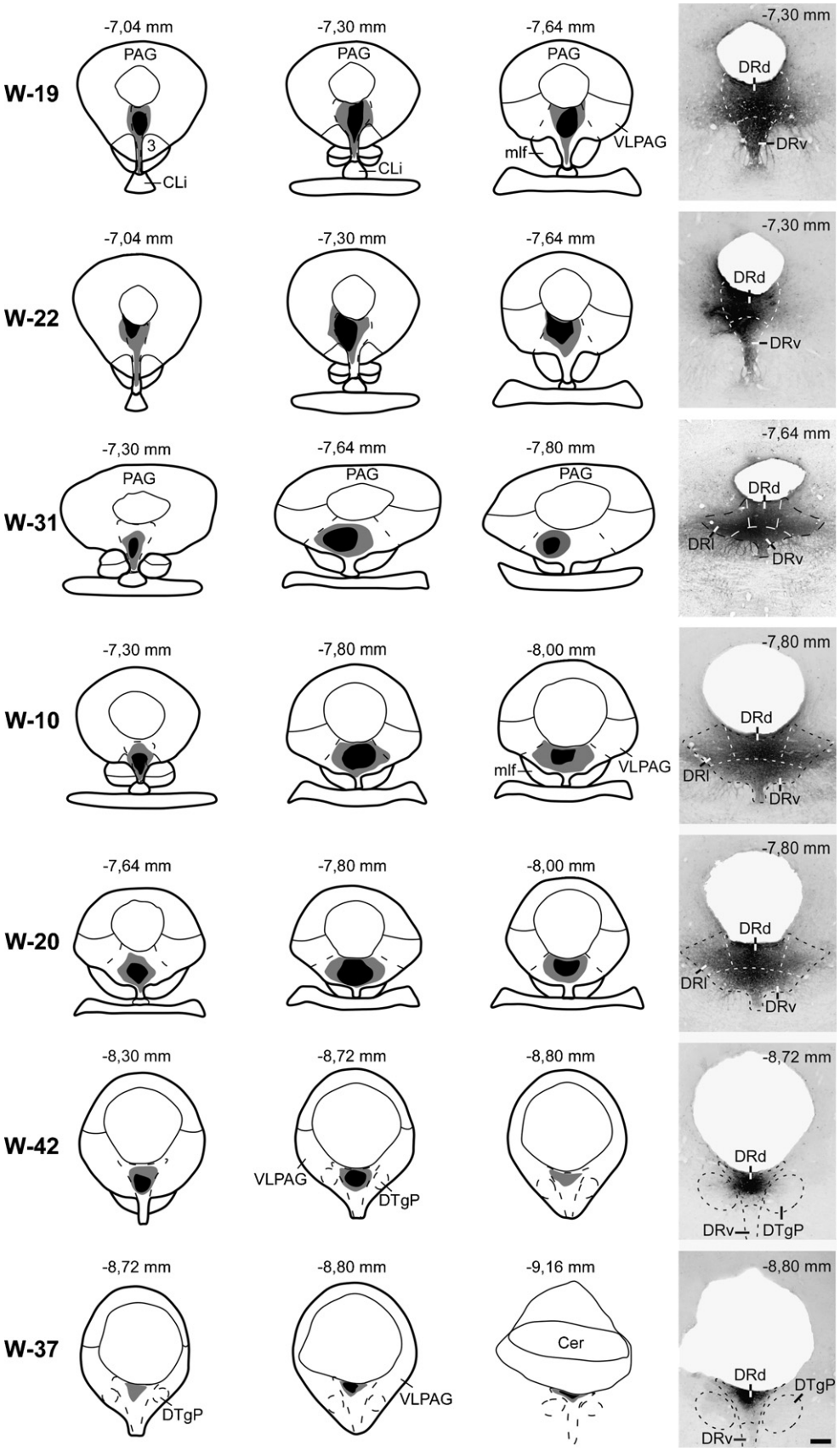

Fig. 1. Schematic drawings illustrating the injections of CTb in the rostral (W-19 and W-22), mid-rostrocaudal (W-10, W-20, and W-31) and caudal parts of the dorsal raphe nucleus (W-37 and W-42), and the respective digital brightfield photomicrographs depicting the center of these injection sites. The black areas indicate regions of dense CTb deposit, and the gray areas the surrounding halo. *Abbreviations*: CLi, caudal linear nucleus of the raphe; DRd, dorsal raphe nucleus, dorsal part; DRl, dorsal raphe nucleus, lateral part; DRv, dorsal raphe nucleus, ventral part; DTgP, dorsal tegmental nucleus, pericentral; VLPAG, ventrolateral periaqueductal gray; PAG, periaqueductal gray; xscp, decussation of the superior cerebellar peduncle. Scale bar = 250 μm.