available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Central amygdaloid axon terminals are in contact with retrorubral field neurons that project to the parvicellular reticular formation of the medulla oblongata in the rat

Toshiko Tsumori[a,1], *Yi Qin*[b,1], *Shigefumi Yokota*[a], *Jian-guo Niu*[a,b], *Yukihiko Yasui*[a,*]

[a]*Department of Anatomy and Morphological Neuroscience, Shimane University School of Medicine, Izumo 693-8501, Japan*
[b]*Department of Anatomy, Ningxia Medical University, Yinchuan 750004, PR China*

ARTICLE INFO

Article history:
Accepted 29 September 2009
Available online 13 October 2009

Keywords:
Amygdala
Retrorubral field
Parvicellular reticular formation
GABAergic
Rat
Orofacial movement

ABSTRACT

The retrorubral field (RRF) contains numerous dopaminergic neurons and projects to the parvicellular reticular formation (RFp) of the medullary and pontomedullary brainstem, where many premotor neurons project to the orofacial motor nuclei. To know how the amygdala affects the RRF–RFp pathway in the rat, we first examined the synaptic organization between the central amygdaloid nucleus (CeA) fibers and the RFp-projecting RRF neurons by using combined anterograde and retrograde tracing techniques. After ipsilateral injections of biotinylated dextran amine (BDA) into the CeA and Fluoro-gold (FG) into the RFp, the prominent overlapping distribution of BDA-labeled axon terminals and FG-labeled neurons was found in the lateral part of the RRF ipsilateral to the injection sites, where the BDA-labeled axon terminals made symmetrical synapses with somata and dendrites of the FG-labeled neurons. Using a combination of retrograde tracing and immunohistochemistry for tyrosine hydroxylase (TH), we secondly demonstrated that the RFp-projecting RRF neurons were immunonegative for TH. Using a combination of anterograde tracing and immunohistochemistry for glutamic acid decarboxylase (GAD), we finally revealed that the CeA axon terminals in the RRF were immunoreactive for GAD. The present results suggest that GABAergic CeA neurons may exert inhibitory influences on non-dopaminergic RRF neurons that project to the RFp in the control of orofacial movements closely related to emotional behavior.

* *Corresponding author.*
E-mail address: yyasui@med.shimane-u.ac.jp (Y. Yasui).

Abbreviations: ACo, anterior cortical amygdaloid nucleus; Amb, ambiguus nucleus; BL, basolateral amygdaloid nucleus; BM, basomedial amygdaloid nucleus; CeA, central amygdaloid nucleus; CPu, caudate-putamen; cp, cerebral peduncle; DEn, dorsal endopiriform nucleus; GP, globus pallidus; I, intercalated amygdaloid nucleus; ic, internal capsule; IO, inferior olive; IP, interpeduncular nucleus; Me, medial amygdaloid nucleus; ml, medial lemniscus; NST, nucleus of the solitary tract; opt, optic tract; PAG, periaqueductal gray; Pir, piriform cortex; PLCo, posterolateral cortical amygdaloid nucleus; Pn, pontine nucleus; PrH, prepositus hypoglossal nucleus; py, pyramidal tract; R, red nucleus; RRF, retrorubral field; SC, superior colliculus; SNr, substantia nigra pars reticulata; SpVe, spinal vestibular nucleus; Sp5, spinal trigeminal nucleus; sp5, spinal trigeminal tract; st, stria terminalis; III, oculomotor nucleus; VII, facial nucleus

[1] T.T. and Y.Q. contributed equally to this work.

0006-8993/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2009.09.118

1. Introduction

The parvicellular reticular formation (RFp) occupies the dorsolateral third of the reticular core in the medullary and pontomedullary brainstem, merging rostrally with the reticular region around the motor trigeminal nucleus (Vm) and caudally with the medullary dorsal reticular nucleus. The RFp is well known to contain many premotor interneurons projecting to the orofacial motor nuclei, such as the motor trigeminal nucleus (Vm), facial nucleus and hypoglossal nucleus (Travers and Norgren, 1983; Ter Horst et al., 1991; Mogoseanu et al., 1993; Li et al., 1995; Yasui et al., 1997; Kolta et al., 2000; Yasui et al., 2004; Yamamoto et al., 2007; Notsu et al., 2008). On the other hand, the retrorubral field (RRF) or nucleus, a midbrain area dorsal and caudal to the substantia nigra pars compacta, is also involved in orofacial motor function (Arts et al., 1998; Uchida et al., 2005). One of the responsible pathways originating from the RRF for the control of orofacial movements is the retrorubroreticular pathway (Arts et al., 1998); the RRF region contains a population of neurons sending their axons to the RFp (von Krosigk and Smith, 1991; von Krosigk et al., 1992). In addition, the central amygdaloid nucleus (CeA), which constitutes an important "emotional–motor" interface (Alheid and Heimer, 1996), participates in the control mechanism of orofacial movements including jaw movements (Mishima et al., 1982; Sasamoto and Ohta, 1982; Ohta and Moriyama, 1986) and projects to the RRF (Gonzales and Chesselet, 1990; Wallace et al., 1989, 1992). Judging from the above, it seems probable that the output signals from the CeA have direct influence on the retrorubroreticular pathway in the control of orofacial movements. However, there have been no studies to examine whether or not RFp-projecting RRF neurons receive monosynaptic inputs from the CeA.

The RRF contains many dopamine neurons which are referred to as the dopaminergic A8 cell group (Dahlström and Fuxe, 1964; Fallon and Loughlin, 1995). Dopamine neurons in the RRF are known to project to several forebrain regions, such as the hippocampal formation (Gasbarri et al., 1996, 1997), amygdala (Deutch et al., 1988; Hasue and Shammah-Lagnado, 2002), bed nucleus of the stria terminalis (Deutch et al., 1988; Hasue and Shammah-Lagnado, 2002), and striatum (Deutch et al., 1988; François et al., 1999). However, it remains unanswered whether or not RFp-projecting RRF neurons are dopaminergic.

Many GABAergic neurons have been found in the CeA by immunohistochemistry for GABA (Nitecka and Ben-Ari, 1987), as well as for glutamic acid decarboxylase (GAD), which is the rate-limiting enzyme in the production of GABA (Mugnaini and Oertel, 1985). Recent studies using *in situ* hybridization of GAD65 mRNA (Poulin et al., 2008) or GAD67 mRNA (Oka et al., 2008) also demonstrated that almost all the CeA neurons are GABAergic. In addition, GABA-immunoreactive CeA axon terminals have been observed not only in the forebrain regions including the parastrial nucleus (Tsubouchi et al., 2007) and posterior lateral hypothalamus (Tsumori et al., 2006), but also in the brainstem regions including the parabrachial nucleus (Jia et al., 2005) and nucleus of the solitary tract (NST) (Jia et al., 1997; Saha et al., 2000). To our knowledge, however, there have been no morphological studies to clarify whether or not the CeA–RRF projection is GABAergic.

In the present study, we first demonstrate the existence of a disynaptic pathway from the CeA to the RFp of the medullary and pontomedullary brainstem via the RRF, using anterograde tracing with biotinylated dextran amine (BDA) combined with retrograde tracing with Fluoro-gold (FG). Secondly, we examine whether or not RFp-projecting RRF neurons are immunoreactive for tyrosine hydroxylase (TH), which catalyzes the rate-limiting step in the synthesis of catecholamines. Finally, we examine whether the CeA projection to the RRF is GABAergic, using anterograde tracing combined with immunohistochemistry for GAD.

2. Results

2.1. *Overlapping distribution and synaptic connections of CeA fibers and RFp-projecting RRF neurons*

2.1.1. *Light microscopic observation*

In this set of experiments, 4 out of 13 operated rats received successful combined injections of BDA into the CeA (Figs. 1A and 2A) and FG into the RFp just ventral to the NST (Figs. 1B and 2B). In these rats, BDA-labeled fibers were distributed bilaterally with a clear-cut ipsilateral dominance in the midbrain tegmental field. At rostral levels of the midbrain, a large number of labeled fibers with bouton-like varicosities were distributed in the substantia nigra pars lateralis as well as in the lateralmost part of the substantia nigra pars compacta, with a small number of labeled fibers in the medial part of the substantia nigra pars compacta as well as in the ventral tegmental area. A few labeled axons were also found in the ventrolateral part of the periaqueductal gray as well as in the deeper layers of the superior colliculus. More caudally, dense plexuses of BDA-labeled fibers were observed throughout the rostrocaudal extent of the RRF; the labeled axon terminals were distributed predominantly in the lateral half and additionally in the medial half of the RRF, and the labeled fibers in the lateral RRF extended into the neighboring tegmental field (Figs. 1C–F). In these levels, some labeled axons were found in the ventrolateral part of the periaqueductal gray and a few labeled axons were seen in the deeper layers of the superior colliculus. FG-labeled neurons, which were small to medium in size and oval or fusiform in shape (Figs. 2C and D and 4B), were also distributed bilaterally with a clear-cut ipsilateral dominance in the midbrain tegmental field. At rostral levels of the midbrain, many FG-labeled neurons were distributed in the dorsolateral part of the substantia nigra pars reticulata and a few labeled neurons were seen in the substantia nigra pars lateralis as well as in the substantia nigra pars compacta. More caudally, large numbers of FG-labeled neurons were distributed throughout the rostrocaudal extent of the RRF; as was the case for the BDA-labeled axons, the labeled neurons were found predominantly in the lateral half and additionally in the medial half of the RRF (Figs. 1C–F). When followed caudally at these levels, FG-labeled tegmental neurons around the RRF increased in number. In the midbrain, FG-labeled neurons were also seen in the deeper layers of the superior colliculus, periaqueductal