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DIRECT BRAIN PROJECTIONS ONTO THE SPINAL GENERATOR OF EJACULATION IN THE RAT

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Abstract—A spinal generator for ejaculation (SGE) has been identified in the rat that orchestrates peripheral events leading to ejaculation. Despite physiological evidence of cerebral influences exerted on the SGE, brain-descending pathways to the SGE have not been fully delineated. A tracing study combining retrograde and anterograde approaches was undertaken in adult male rats in order to identify brain sites containing neurons that directly project onto SGE neurons. Fluorogold (FG) was microinjected as a retrograde tracer into the SGE area in the central medial gray of the third lumbar (L3) spinal segment. FG-positive neurons were found in various structures in medulla oblongata, pons, and forebrain. Among the brain structures already known as participating in the brain control of ejaculation and harboring retrogradelly-labeled neurons, the ventrolateral part of the gigantocellular nucleus and the raphe pallidus/magnus in medulla oblongata as well as the lateral hypothalamus were targeted with the anterograde tracer dextran amine (DA). Galanin and substance P receptor (NK1) were used as markers of SGE neurons. DA-positive fibers and varicosities originating in the targeted brain sites were found to make close appositions with neurons expressing galanin or NK1 receptors in central medial gray of L3–L4 spinal segments. This study provides new insights regarding the anatomical support for the brain control of ejaculation via direct influences onto the SGE.

Key words: ejaculation, gigantocellular nuclei, lateral hypothalamus, Lumbar spinal cord, raphe nuclei, rat.

INTRODUCTION

Ejaculation consists of two distinct successive phases (emission and expulsion). Emission and expulsion result from coordinated activation of autonomic and somatic neural pathways. Several lines of evidence suggest that coordination takes place at the spinal level where a group of cells has been identified as playing a role of generator for ejaculation (Truitt and Coolen, 2002; Truitt et al., 2003; Borgdorff et al., 2008). Results from a series of neuroanatomical, tracing, and functional studies in adult rats (Ju et al., 1987; McKenna and Nadelhaft, 1986; Nicholas et al., 1999; Coolen et al., 2003; Borgdorff et al., 2008; Sakamoto et al., 2008; Sun et al., 2009; Truitt and Coolen, 2002; Xu et al., 2005, 2006) focusing on this spinal generator of ejaculation (SGE) can be summarized as follows: (i) SGE is mainly located around the central canal (Rexed's areas X and VII medial) at the third and fourth lumbar (L3–L4) spinal segments; (ii) SGE is constituted of neurons sending projections to the parvocellular part of the subparafascicular nucleus of the thalamus (neurons referred as lumbar spinothalamic (LSt)) and to the autonomic and somatic spinal nuclei commanding the peripheral events of ejaculation; (iii) afferent fibers conveying sensory inputs mediating ejaculation terminate in the SGE area; (iv) SGE harbors interneurons that contain the neuropeptides galanin, cholecystokinin, enkephalin, gastrin-releasing peptide and express neurokinin-1 (NK1), NMDA, and androgen receptors. Activity of the SGE is dependent on cerebral facilitation or disinhibition. A cerebral network involved in ejaculation has been delineated in the male rat (for review see Coolen, 2005; Giuliano and Clément, 2012). It comprises groups of neurons in subdivisions of the medial amygdala (posterodorsal part), the thalamus (parvicellular subparafascicular nucleus), the stria terminalis (posteromedial bed nucleus), the hypothalamus (posterodorsal and medial preoptic nuclei, paraventricular nucleus, and lateral hypothalamus), and the pons (raphe and gigantocellular nuclei). In particular, it has been demonstrated that a complete ejaculatory response (i.e. coordinated emission and expulsion phases) can be induced by intracerebral delivery of dopamine agonists in anaesthetized male rats (Clément et al., 2007, 2006). The medial preoptic area of the hypothalamus (MPOA) is likely involved in mediating such a pro-ejaculatory effect of dopamine

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Abbreviations: 5-HT, serotonin; DA, dextran amine; DNS, donkey normal serum; FG, fluorogold; Gi, gigantocellular nucleus; GiA, gigantocellular nucleus pars alpha; GiV, gigantocellular nucleus ventral part; Givl, ventrolateral division of the gigantocellular nucleus; LH, lateral hypothalamus; LPGi, lateral paragigantocellular nucleus; LSt, lumbar spinothalamic; M1, primary motor cortex; MPOA, medial preoptic area of the hypothalamus; NK1, neurokinin-1; PAF, Paraformaldehyde; PBS, Phosphate-buffered saline; PVN, paraventricular nucleus of the hypothalamus; Red, red nucleus; RMg, raphe magnus; ROb, raphe obscurus; ROI, region of interest; RPa, raphe pallidus; SGE, spinal generator for ejaculation.

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agonists (Hull et al., 1989; Kitrey et al., 2007). It is however well established that MPOA does not directly project to the spinal cord, meaning that the final excitatory output to SGE originates in other brain site(s) in direct connection with MPOA (notably the paraventricular nucleus). Brain inhibitory influence on ejaculation can also be inferred from numerous studies in the rat. As a whole the data indicate that serotonergic neurons lying in the ventrolateral medulla modulate the expulsion reflex through, at least in part, synapses onto lumbosacral (L6-S1) interneurons (Marson and McKenna, 1996; Johnson and Hubscher, 1998; Gravitt and Marson, 2007). However, it is unclear if SGE is under such an inhibitory tone although projections from pontine structures (including gigantocellular and caudal raphe nuclei) to SGE neurons have been reported (Coolen et al., 2004). Overall, evidence for the existence of brain projections onto SGE neurons is scarce. The present study was undertaken in order to delineate direct brain-descending projections onto SGE neurons by coupling retrograde and anterograde tracing in the male rat.

Part of the results of this work have been presented in oral form at the 14th meeting of the International Society for Sexual Medicine, Seoul, Korea, September 2010 and an abstract has been published (Clément et al., 2010).

EXPERIMENTAL PROCEDURES

Animals

All animal experiments were carried out in accordance with the European Community Council Directive (86/609/EEC) on the use of laboratory animals. Fifteen adult male sexually naïve Wistar rats (Janvier, St Genest L'Isle, France) weighing 250–275 g were kept for at least 6 days in our animal facilities in standardized environmental conditions (lights off from 9.00 a.m. to 9.00 p.m., temperature 22 ± 1 °C, with water and standard laboratory food *ad libitum*) before use. All efforts were made to minimize the number of rats used and their suffering.

Dye intraspinal microinjections – retrograde tracing

Rats (n = 4) were anaesthetized with pentobarbital (40 mg/kg i.p.) and their spine was exposed dorsally and fixed in a stereotaxic frame. Laminectomy between vertebrae lumbar (L1) and thoracic (T13) exposed spinal segment L3–L4. After dura removal, the spinal cord was incubated 20 min with 3 units/ml collagenase type VII from Clostridium histolyticum (Sigma–Aldrich, St Quentin Fallavier, France) to improve access to spinal tissue. Then a glass micropipette (tip diameter ~70 µm) was set in a micromanipulator apparatus. The tip of the micropipette was placed on the spinal cord dorsal surface, adjacent to the dorsal spinal artery, and lowered vertically to 1700-µm depth for targeting SGE in lumbar central gray area (Rexed's laminae X and VII medial). A volume of 0.2 µl of 2% hydroxystilbamidine in isotonic saline solution (fluorogold; FG, Interchim, Montluçon, France) was delivered over 2 min using a hydraulic microdriving system (Trent-Wells, Coulterville, CA, USA). At the end of the injection, the micropipette was left in place for 5 min and then slowly removed. The area of laminectomy was filled with agar solution to protect the spinal tissue and overlying muscles and skin were sutured. Animals were housed individually for 14 days until sacrifice for histological procedure.

Dye intracerebral microinjection – anterograde tracing

Rats (n = 9) were anaesthetized with pentobarbital (40 mg/kg i.p.) and their heads held in a stereotaxic frame. A small burr hole was made and a microinjection cannula (28G outer diameter) was lowered using a micromanipulator into one of the three brain regions selected on the basis of retrograde tracing experiment results. Brain sites were targeted using the following coordinates (according to Paxinos and Watson's rat brain atlas, 1998):

(i) 10 mm posterior to Bregma, 0.4 mm lateral to midline, 10.5 mm below the skull surface; corresponding to raphe magnus (RMg) and raphe pallidus (RPa) nuclei, thereafter referred as RMg/RPa.
(ii) 11.5 mm posterior to Bregma, 1.2 mm lateral to midline, 10.5 mm below the skull surface; corresponding to the border between gigantocellular reticular (Gi), ventral part of the gigantocellular reticular nucleus (GiV), and lateral paragigantocellular (LPGi) nuclei, thereafter referred as ventrolateral Gi (Givl).
(iii) 3.5 mm posterior to Bregma, 1.5 mm lateral to midline, and 8.4 mm below skull surface; corresponding to the lateral hypothalamus (LH).

A volume of 0.8 µl of 5% dextran amine conjugated to Texas Red (DA, 10,000 MW; Invitrogen, Paisley, UK) in sterile water was injected over 4 min using a hydraulic microdriving system. At the end of the injection, the micropipette was left in place for 10 min and then slowly removed. Animals were housed individually for 14 days until sacrifice for histological procedure.

Histological procedure

Rats were anaesthetized with pentobarbital (60 mg/kg, i.p.) and transcardially perfused with phosphate-buffered saline 0.01 M, pH = 7.4 (PBS) and then paraformaldehyde 4% (PAF; in PBS). Lumbar spinal cord (L1–L6 spinal segments) and brain were gathered, post-fixed for 2 h in PAF, placed in 30% sucrose for 48 h and then frozen by immersion in isopentane (3 min, −40 °C) and stored at −80 °C until sectioned.

Serial coronal 30-µm-thick spinal cord and brain sections were performed using a cryostat then collected on Superfrost plus Gold® adhesion microscope slides (Menzel-Glaser, Braunschweig, Germany) and stored at −20 °C until coloration/immunohistochemical procedure or mounting.

For retrograde tracing, one series of brain and spinal cord sections was mounted in Vectashield® medium (Vector laboratories, Burlingame, CA, USA) for