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Opioid receptor and β-arrestin2 densities and distribution change after sexual experience in the ventral tegmental area of male rats

René Garduño-Gutiérrez[a,b], Martha León-Olea[b], Gabriela Rodríguez-Manzo[a,*]

[a] Departamento de Farmacobiología, Cinvestav Sede Sur, Calzada de los Tenorios 235, Ciudad de Mexico 14330, Mexico
[b] Dirección de Neurociencias, Instituto Nacional de Psiquiatría Ramón de la Fuente, Av. México-Xochimilco 101, Ciudad de Mexico 14370, Mexico

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ABSTRACT

Sexual experience modifies brain functioning and copulatory efficiency. Sexual activity, ejaculation in particular, is a rewarding behavior associated with the release of endogenous opioids, which modulate the activity of the mesolimbic dopaminergic system (MLS). In sexually exhausted rats, repeated ejaculation produces μ (MOR) and δ opioid receptor (DOR) internalization in ventral tegmental area (VTA) neurons, as well as long-lasting behavioral changes suggestive of brain plasticity processes. We hypothesized that in sexually naïve rats the endogenous opioids released during sexual experience acquisition, might contribute to brain plasticity processes involved in the generation of the behavioral changes induced by sexual experience. To this aim, using double immunohistochemistry and confocal microscopy, we compared in vivo MOR, DOR and β-arrestin2 densities and activation in the VTA of sexually naïve males, sexually experienced rats not executing sexual activity prior to sacrifice and sexually experienced animals that ejaculated once before sacrifice. Results showed that sexual experience acquisition improved male's copulatory ability and induced persistent changes in the density, cellular distribution and activation of MOR and β-arrestin2 in VTA neurons. DOR density was not modified, but its cellular location changed after sexual experience, revealing that these two opioid receptors were differentially activated during sexual experience acquisition. It is concluded that the endogenous opioids released during sexual activity produce adjustments in VTA neurons of sexually naïve male rats that might contribute to the behavioral plasticity expressed as an improvement in male copulatory parameters, promoted by the acquisition of sexual experience.

1. Introduction

Sexual behavior in rodents is an innate, highly stereotyped behavior involving three different motor responses: mounting behavior, intromissive behavior and ejaculatory behavior [1]. In spite of its innate nature, sexual behavior display can be improved and becomes optimal with experience. Male rats exposed for the first time to a sexually receptive female take a long time investigating the couple before starting mating. They also exhibit a larger number of mounts and intromissions and require a longer time to achieve ejaculation as compared to experienced animals. Experience improves copulatory efficiency reducing all these variables and stabilizing the copulatory pattern [2–4]. Sexual experience also augments the olfactory interest of male rodents in female sexually related chemosignals [5]. These behavioral changes indicate that the males learn to associate the sexual experience with the cues that predict it.

There is evidence for differences in brain functioning related to sexual experience. For instance, sexual experience enhances neuronal activation of the male rat vomeronasal pathway [6], which is involved in the detection of odors from sexually receptive females [7]. Also, estrous female odors increase Fos protein expression in brain regions receiving projections from the vomeronasal pathway, i.e. the medial preoptic area (mPOA) and the bed nucleus of the stria terminalis (BNST), or in the reward circuit, specifically in the nucleus accumbens (NAcc), of sexually experienced, but not of sexually naïve animals [8]. In line with this finding, ejaculation activates more cells within the mPOA [9] and the NAcc [10] of sexually experienced male rats as compared to sexually naïve animals copulating for the first time. Sexual experience also modifies neuronal structure and proliferation. Thus, mating experience increases dendritic spine density in the medial prefrontal cortex pyramidal neurons and in the granular neurons of the dentate gyrus of the hippocampus [11]. In this last structure, both acute and chronic sexual experience, promotes neurogenesis and stimulates dendritic spine growth [12]. These neural changes evidence the occurrence of experience-induced synaptic plasticity.

Sexual behavior is rewarding [13] and, like other natural rewards,

* Corresponding author at: Departamento de Farmacobiología, Cinvestav Sede Sur, Calz. De los Tenorios 235, Col. Granjas Coapa, Deleg. Tlalpan, Ciudad de México 14330, Mexico.
E-mail address: grodrigu@cinvestav.mx (G. Rodríguez-Manzo).

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e.g. food intake, it is processed by the mesolimbic dopaminergic system (MLS) [14]. The rewarding nature of male sexual activity appears to play a role in the sexual experience-induced improvement of copulatory ability [13]. With experience, animals engage more rapidly in sexual activity, ejaculate more often and show shorter postejaculatory intervals (reviewed in [2]); changes that can be interpreted as experience-related increases in sexual interest. The MLS also shows enhanced neuronal responses in sexually experienced male rats as compared to sexually naïve animals. Thus, exposure to an inaccessible receptive female produces higher Fos expression in the NAcc of sexually experienced males, as compared with sexually naïve male rats [10] and it has been postulated that natural reinforcers, like sexual reward, are capable of leading to behavioral and neurochemical plastic changes [15].

The rewarding properties of ejaculation have been associated with the activation of brain opioid systems [4]. For instance, ejaculation induces a naloxone-reversible reward state, indicative of the release of endogenous opioids [16]. In line with this finding, ejaculation increases μ (MOR) and δ opioid receptor (DOR) internalization in VTA neurons of male rats, pointing to their activation by the released endogenous opioids [17]. Moreover, copulation to ejaculation increases enkephalins' content in the hypothalamus, midbrain and frontal cortex of sexually experienced rats [18].

Endogenous opioids indirectly modulate the activity of the MLS by acting at MOR located on GABA neurons [19,20,21] and GABAergic terminals at the VTA [22]. Recently, endogenous opioids have also been postulated to directly activate dopaminergic and non-dopaminergic VTA neurons [23]. Previous data from our laboratory showed that MOR internalization in VTA neurons was maximal in males that ejaculated repeatedly until becoming sexually exhausted [17]. Since the sexually exhausted rat is characterized by exhibiting long lasting behavioral and physiological changes, suggestive of the occurrence of brain plasticity processes [24], we hypothesized that the endogenous opioids released during copulation and acting at the MLS, might contribute to neuronal plasticity processes resulting from sexual experience acquisition.

On these bases, we decided to explore if VTA opioid receptor density and activation were modified by sexual experience. To this aim, we compared in vivo MOR and DOR densities and activation in the VTA of sexually naïve males, sexually experienced rats not executing sexual activity prior to sacrifice and sexually experienced animals that ejaculated once before sacrifice. Double immunohistochemistry and confocal microscopy were used to determine MOR, DOR or β-arrestin2 densities, and their co-localization as an indicator of opioid receptor activation.

2. Materials and methods

2.1. Animals

Fourteen adult male Wistar rats (250–300 g, b.w.) were group housed in acrylic boxes for the duration of the experiment. The colony room was maintained at 22 °C on a 12 h/12 h reversed light–dark cycle (lights off at 10:00 h). Food and water were available ad libitum. Female Wistar rats served as sexual stimuli. Sexual receptivity was induced by the sequential s.c. injection of estradiol benzoate (4 μg/rat, Sigma-Aldrich, St. Louis, MO) and progesterone (2 mg/rat, Sigma-Aldrich, St. Louis, MO) 48 h and 4 h before the sexual behavior tests, respectively.

The Local Committee of Ethics on Animal Experimentation approved all experimental procedures, which followed the regulations established in the Mexican official norm for the use and care of laboratory animals NOM-062-ZOO-1999. This norm follows the principles of the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Sexual behavior tests

Sexual behavior tests were performed during the dark phase of the cycle under red dim light. Briefly, male rats were placed into polycarbonate cylindrical arenas (40 cm diameter, 60 cm height) with the floor covered with fine sawdust, and after a 5-min adaptation period, a receptive female was introduced and copulation to ejaculation was allowed. Male rats were rendered sexually experienced by exposing them to five independent sexual behavior tests with a receptive female, run every other day. Those animals achieving ejaculation in < 15 min, in at least three of the tests, were considered sexually experienced.

2.3. Tissue preparation

Animals were deeply anesthetized with sodium pentobarbital (200 mg/kg, i.p.) and intracardially perfused with 250 ml sodium chloride (0.9%) and heparin (0.2%), followed by 400 ml paraformaldehyde (4%) in a phosphate sodium buffer, supplemented with 0.9% sodium chloride (PBS) 0.1 M, pH 7.3. The brains were removed from the skull and post-fixed for 1 h at 4 °C. At the end, brains were placed in a cryoprotectant solution (sucrose at 30% in sodium phosphate buffer 0.1 M) and stored at 4 °C until further use. Twenty-four 30 μm coronal sections of the brain region containing the VTA (Coordinates: −5.2 to −6.3 anterior to bregma; [36] were cut using a freezing microtome (Kriomat 1700, Leica, Germany).

2.4. Immunohistochemistry

2.4.1. Double immunofluorescence for MOR or DOR and β-arrestin2

The immunohistochemical procedures followed were reported earlier [17]. Briefly, tissue sections were washed and incubated with a blocking solution. Half of the sections were incubated with the rabbit anti-MOR antibody (Calbiochem, PC 165L; [26]) diluted 1:100, together with the goat anti- β-arrestin2 antibody (Santa Cruz, SC-6387, [27]) diluted 1:100 at 4 °C, for the detection of MOR and β-arrestin2. The other half of the sections were incubated with goat anti-DOR antibody (Santa Cruz, SC-7492; [28]) diluted 1:100, together with the mouse anti-β-arrestin2 antibody (Santa Cruz, SC-13140, [29]) diluted 1:100 for the detection of DOR and β-arrestin2. After washing, the sections were incubated with the secondary antibodies. The antibodies used were rhodamine donkey anti-rabbit (Jackson Immunoresearch, 711-026-152), diluted 1:100; FITC donkey anti-goat (Jackson Immunoresearch, 705-096-147) diluted 1:100 for detection of MOR and β-arrestin2, respectively. For the detection of DOR and β-arrestin2, the secondary antibodies used were Alexa 488 donkey anti-goat (Molecular probes, diluted 1:300) and Alexa 555 donkey anti-mouse (Molecular Probes, diluted 1:100), respectively. Immunohistochemical controls lacked the first antibodies.

2.4.2. Quantitative analysis

Immunodetection of MOR plus β-arrestin2 and DOR plus β-arrestin2 was performed in coronal brain sections (24 for each rat), using half of them for MOR and the other half for DOR detection. Consecutive serial sections were alternated for MOR and DOR immunohistochemical analysis in three animals of each experimental group. The sections were analyzed with a Zeiss META 510 laser scanning confocal microscope, equipped with an Ar laser/488 nm emissions (for FITC and Alexa 488) and a He−Ne laser/543 nm emissions (for rhodamine and Alexa 555), attached to an Axiovert 200 M microscope with a 63X PlanApo 1.4 na objective (Carl Zeiss). Images were collected without zooming. Before sample analysis, we conducted a lambda stack to obtain the emission spectrum of each fluorophore (rhodamine, FITC, Alexa 488, Alexa 555). For the analysis of the samples a scan with the Ar laser/488 nm was conducted followed by a second scan using the He−Ne laser/543 nm (multitracking acquisition). Optical areas of 20,449 μm^2, with a thickness of 0.45 μm in the Z axis were bilaterally analyzed in all sections. MOR and β-arrestin2, DOR and β-arrestin2 immunoreactivities (ir), as well as their respective co-localization were obtained, separated into three different channels (channel 1 for FITC or Alexa 488, channel 2 for rhodamine or Alexa 555, and channel 3 for their co-localization) within