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Research report

Involvement of dopamine in the differences in sexual behaviour between Roman high and low avoidance rats: An intracerebral microdialysis study

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HIGHLIGHTS

- RHA rats show higher sexual motivation and better copulatory performance than RLA rats.
- Repeated sexual activity does not abolish these differences between RHA and RLA rats.
- This occurs together with a greater dopamine release in the accumbens of RHA vs. RLA rats.
- A lower dopaminergic tone in RLA vs. RHA rats may explain their different sexual patterns.

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ABSTRACT

Outbred Roman high- (RHA) and low-avoidance (RLA) rats are selected for respectively rapid vs. poor acquisition of the active avoidance response and display different copulatory patterns when exposed to a sexually receptive female, with RHA rats showing more robust sexual motivation and better performance than RLA rats also after repeated sexual activity. Here we show that the distinct patterns of sexual behaviour of the Roman lines are correlated with differences in the activity of the dopaminergic mesolimbic system, which plays a key role in sexual motivation and copulatory performance. Thus, differential increases in the concentrations of dopamine and its main metabolite 3,4-dihydroxyphenylacetic acid, occurred in dialysates obtained from the nucleus accumbens shell of naïve and sexually experienced Roman rats during the anticipatory and consummatory phases of sexual activity. These differences were particularly evident between sexually naïve RHA and RLA rats and tended to diminish but still persisted between sexually experienced rats, as did the differences in sexual behaviour. Analysis of the biochemical and behavioural findings showed that, while in RHA rats sexual experience caused a shift in the changes in both the dopaminergic activity and copulation towards the first period of the sexual test, in RLA rats sexual experience increased dopaminergic activity and copulation throughout the entire test. Therefore, this study adds experimental support to the view that the different sexual patterns of the Roman lines are due, at least in part, to a more robust functional tone of the mesolimbic dopaminergic system of RHA rats.

1. Introduction

The Roman high- (RHA) and low-avoidance (RLA) outbred rat lines, originally selected for respectively rapid vs. poor acquisition of the active avoidance response in a shuttle-box [1–5] display different copulatory patterns with a sexually receptive female rat, with RHA rats showing higher sexual motivation and better

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copulatory performance than RLA rats [6]. These differences are more pronounced during the first copulation test, as reflected by marked differences in several copulatory parameters between the two lines. Thus, more than 80% of RHA rats vs. only 40% of RLA rats engage in mounts/intromissions and ejaculate in the first copulation test. These differences persist, although attenuated, after repeated copulation tests [6]. Interestingly, the different copulatory patterns of RHA and RLA rats are differentially affected by the dopamine agonist apomorphine and the dopamine antagonist haloperidol given alone or in combination at doses that facilitate and inhibit, respectively, copulatory behaviour [7]. In particular, RLA rats are more responsive than RHA rats to the facilitating effects of apomorphine and to the inhibitory effects of haloperidol on sexual behaviour, as indicated by the greater changes induced by these drugs especially at the lower doses in several copulatory parameters in RLA rats compared to RHA and Sprague Dawley (SD) rats, used as a genetically heterogeneous reference strain. Notably, dopamine facilitates both the anticipatory (sexual motivation/arousal) and the consummatory (penile erection and copulation) phases of sexual behaviour in laboratory animals and humans, by acting in the medial preoptic area and hypothalamic nuclei [8–17] and in mesolimbic brain areas (i.e., ventral tegmental area and nucleus accumbens) [18–22].

Together, the above results raise the possibility that a differential functional dopaminergic tone may be responsible for the different copulatory patterns of RHA and RLA rats. Accordingly, a lower availability of inhibitory D2 autoreceptors (measured by dopamine receptor binding and mRNA assays) has been recently reported in the substantia nigra/ventral tegmental area, caudate putamen and nucleus accumbens of RHA vs. RLA rats [23], which may account for a more robust dopaminergic tone in RHA rats as compared with their RLA counterparts. To test this hypothesis, we studied the activity of the mesolimbic dopaminergic system by measuring extracellular dopamine and its main metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in dialysates from the shell compartment of the nucleus accumbens (NAs) of sexually naïve (i.e., never exposed to a receptive female) and sexually experienced (i.e., submitted to five preliminary copulation tests) RHA and RLA rats during the anticipatory and consummatory phases of sexual behaviour.

2. Materials and methods

2.1. Animals

Outbred Roman high- (RHA) and low-avoidance (RLA) male rats ($N = 30$ for each rat line, 300–350 g at the beginning of the experiments) were from the colony established in 1998 at the University of Cagliari, Italy [5]. The procedures used for the selective breeding of the Sardinian colony have been described in detail elsewhere [24].

Ovariectomized stimulus SD female rats (250–330 g at the beginning of the experiments) used in all the experiments, were obtained from Harlan Nossan (Correzzana, Italy). Animals were kept 4 per cage (38 cm × 60 cm × 20 cm) and were acclimated to the housing facilities of the Department of Biomedical Sciences of the University of Cagliari for at least 10 days before the beginning of the experiments under controlled environmental conditions (24 °C, 60% humidity, reversed 12 h light/dark cycle, with lights off from 08:00 to 20:00 h) and with water and standard laboratory food ad libitum. To limit the stress due to manipulation during the experiments, each animal was daily handled for approximately 1–2 min throughout the habituation period; in addition, contact with the animal house maintenance personnel was limited to a single attendant and bedding in the home cages was never changed either the day before or on the day of the experiment. The experiments were performed between 10:00 and 18:00 h according to the guidelines of the European Communities Directive of September 22, 2010 (2010/63/EU) and the Italian Legislation (D.L. March4, 2014, no. 26), and were approved by the Ethical Committee for Animal Experimentation of the University of Cagliari.

2.2. Experimental groups

Sexually naïve rats were adult male RHA and RLA rats never exposed to a sexually receptive female (ovariectomized, oestradiol + progesterone primed), whereas sexually experienced rats were previously exposed to five consecutive classical copulation tests at three day intervals with a receptive female rat [6,7]. Oestrus was induced by subcutaneous injections of oestradiol benzoate (200 μg/rat in peanut oil) and progesterone (0.5 mg/rat in peanut oil), 48 and 6 h before the behavioural tests, respectively, and ascertained by May–Grunwald–Giemsa colouration and microscopical examination of vaginal smears 1 h before the experiments. The five preliminary copulation tests were used to make male rats of the two lines sexually experienced, e.g., able to show a constant level of copulatory activity when put together with a receptive female. Two days after these tests, sexually experienced male Roman rats that satisfied the criterion of at least one ejaculation reached in each of the last two tests were included in the microdialysis studies and underwent stereotaxic surgery for microdialysis probe implantation.

2.3. Microdialysis in the NAs during sexual behaviour

The day before the microdialysis experiment, naïve or sexually experienced RHA and RLA rats were stereotaxically implanted (Stoelting Co., Wood Dale, IL, USA), under isoflurane anaesthesia (1.5–2%) (Harvard Apparatus, Holliston, MA, USA), with a microdialysis probe with a *U*-shaped dialysis membrane (approximately 2 mm of free surface for dialysis), prepared as previously described [15], and aimed unilaterally at the NAs (coordinates: 1.8 mm anterior and 0.8 mm lateral to bregma, and 7.8 mm ventral to dura) [25]. The day of the experiment, during the dark phase of the cycle, the rats were transferred to a mating cage (45 cm × 30 cm × 24 cm) located in a sound proof room lit by a dim red light. The mating cage contained another small Plexiglas cage (15 cm × 15 cm × 15 cm) with 25 holes (Ø 2 mm) in each vertical wall to allow for visual, olfactory and acoustic interaction. After a 2 h habituation period, the microdialysis probe was connected via polyethylene tubing to a CMA/100 microinfusion pump (Harvard Apparatus, Holliston, MA, USA) and perfused with Ringer's solution, containing 147 mM NaCl, 3 mM KCl and 1.2 mM $CaCl_2$, pH 6.5, at a constant flow rate of 2.5 μl/min. After a 2 h equilibration period, dialysates were collected throughout the experiment every 15 min in aliquots of 37.5 μL contained in polyethylene tubes that were kept on ice for the determination of dopamine and DOPAC concentrations, as described below. After collecting at least four dialysate aliquots, a sexually receptive female rat was introduced into the small cage located inside the mating cage for 30 min, during which two more dialysate aliquots were collected. In these conditions male rats that can see, hear and smell the female but cannot touch or directly interact with her, show non contact erections (see below). After this period, the small cage was removed from the mating cage and sexual interaction/copulation was allowed for 75 min, during which five more dialysate aliquots were collected. At the end of this period, the female was then removed from the mating cage and an additional dialysate aliquot was collected [15,18,21]. Sexual parameters related to the anticipatory and consummatory phases of sexual behaviour were recorded throughout the experiment (see below).