Behavioural Brain Research 334 (2017) 129–134

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Modulation of the storage of social recognition memory by neurotransmitter systems in the insular cortex

Lorena E.S. Cavalcante[a,b], Carolina G. Zinn[a,b], Scheila D. Schmidt[a,b], Bruna F. Saenger[a,b], Flávia F. Ferreira[a,b], Cristiane R.G. Furini[a,b], Jociane C. Myskiw[a,b,*], Ivan Izquierdo[a,b,*]

[a] Memory Center, Brain Institute, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, RS, 90610-000, Brazil
[b] National Institute of Translational Neuroscience, National Research Council, both at Av. Ipiranga, 6690 – IPB – 2nd floor, HSL, 90610-000, Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
Social recognition memory
Insular cortex
Modulatory systems
Dopamine receptors
β-adrenergic receptors
5-HT_{1A} receptors

ABSTRACT

The insular cortex (IC) receives projections from prefrontal, entorhinal and cingulate cortex, olfactory bulb and basal nuclei and has reciprocal connections with the amygdala and entorhinal cortex. These connections suggest a possible involvement in memory processes; this has been borne out by data on several behaviors. Social recognition memory (SRM) is essential to form social groups and to establish hierarchies and social and affective ties. Despite its importance, knowledge about the brain structures and the neurotransmitter mechanisms involved in its processing is still scarce. Here we study the participation of NMDA-glutamatergic, D1/D5-dopaminergic, H2-histaminergic, β-adrenergic and 5-HT_{1A}-serotoninergic receptors of the IC in the consolidation of SRM. Male Wistar rats received intra-IC infusions of substances acting on these receptors immediately after the sample phase of a social discrimination task and 24 h later were exposed to a 5-min retention test. The intra-IC infusion of antagonists of D1/D5, β-adrenergic or 5-HT_{1A} receptors immediately after the sample phase impaired the consolidation of SRM. These effects were blocked by the concomitant intra-IC infusion of agonists of these receptors. Antagonists and agonists of NMDA and H2 receptors had no effect on SRM. The results suggest that the dopaminergic D1/D5, β-adrenergic and serotonergic 5-HT_{1A} receptors in the IC, but not glutamatergic NMDA and the histaminergic H2 receptors, participate in the consolidation of SRM in the IC.

1. Introduction

Memories are not stored in single sites, but in parallel networks comprising several sites of the brain, whose hub is usually the hippocampus [1–9]. Storage sites have been described in the basolateral amygdala [1,2], septum [2], and various neocortical areas [10–12] depending on the task. Their joint activation is believed to determine memory strength [1,13]. In the posttraining period, extrahippocampal cortical storage sites usually enter into play 1 h or more after the hippocampus and amygdala [3,5,6,9]. This delay may be explained by the successive build-up of biochemical processes related to storage in the cortical projecting sites [4,6,9]. The joint entry into play of the extrahippocampal multisynaptic networks is slower in the posttraining period (1–6 h) than at the time of retrieval, when it is quick and simultaneous perhaps because it does not involve protracted biochemical correlates [14,15]. The participation of the several parallel storage and retrieval sites varies with the memory under study [6,10–12].

The olfactory bulbs, anterior olfactory nucleus, medial prefrontal cortex, medial amygdala nucleus, basolateral amygdala, ventral hippocampal CA1 region and dorsal hippocampus play a role in the memory consolidation of social recognition in rodents [16,17]. This was determined by a variety of procedures regional pCREB, c-Fos and Arc measurements, ablation studies, and most importantly, by optogenetic methods [19] and by microinfusions of putative neurotransmitter agonists and antagonists into those brain areas [20]. These last two methods are decisive to determine the participation of a given neural ensemble in a behavioral task. Microinjections determine the structure; optogenetics determine the cell type involved. Drugs acting on known transmitter receptors, particularly antagonists, are classically taken to give indications as to the synaptic receptors involved in the brain area under study [1–6,13,21,22].

The insular cortex (IC) is anatomically linked to the structures that underlie most types of recognition learning [23–26]. Here we explore whether the IC could also play a role in social recognition memory (SRM), a major type of recognition memory at the core of all social behavior both in animals and humans [16,17,27]. We used basically the same approach that recently showed [20] that the basolateral amygdala and hippocampus are involved in SRM; namely, the localized

* Corresponding authors at: Av. Ipiranga, 6690 – IPB – 2nd floor, HSL – Pontifical Catholic, University of Rio Grande do Sul (PUCRS), Porto Alegre, RS, 90610-000, Brazil.
E-mail addresses: jociane_carvalho@hotmail.com (J.C. Myskiw), izquier@terra.com.br (I. Izquierdo).

http://dx.doi.org/10.1016/j.bbr.2017.07.044
Received 22 April 2017; Received in revised form 26 July 2017; Accepted 27 July 2017
Available online 29 July 2017

microinfusion of drugs known to act on specific memory modulatory receptors into the structure to be studied. Here we study the influence of agonists and antagonists of NMDA, D1/D5, H2, β-noradrenergic and 5-HT_{1A} receptors in the IC on the storage of SRM memory. Evidence suggests a role for the IC in the memory consolidation of several tasks [24,26,28–31], including recognition [32]. In humans, deficits in aspects of emotional cognition have been described in patients with insular damage [33,34] and fMRI changes in the IC have been observed in the recognition of disgust faces [34], but these data fall short of suggesting an involvement in social recognition.

Recognition memory is based on the ability to assess the familiarity of a stimulus previously encountered, that is, the ability to distinguish between familiarity and novelty [17,24,35]. There are, however, several types of recognition memory, each possibly involving to an extent different networks. In particular, social recognition memory (SRM) is essential for adaptive social behavior and can be studied in rodents, by measuring their natural tendency to explore the odor of an unfamiliar conspecific more persistently than that of a familiar one [17,20,36,37]. Disruption of social recognition underlies important disease entities [38]. Recognition memory in rodents and many other animals relies on odor memory, whereas in humans it depends largely on visual cues.

Blockade of dopaminergic receptors in the IC or amygdala impairs olfactory or gustatory learning; blockade of β-adrenergic receptors in IC hinders consolidation of inhibitory avoidance [30,31]. The posttraining blockade of 5-HT_{1A} receptors in the IC impairs memory consolidation of the inhibitory avoidance task [29]. These findings suggest that classic neurotransmitters have an important role in the formation of other types of memory in IC, but there is a lack of knowledge about the role of the receptors involved in IC in SRM. Here we investigate the participation of glutamatergic NMDA, dopaminergic D1/D5, histaminergic H2, 5-HT_{1A} and β-adrenergic receptors in the IC on the consolidation of SRM by infusing their agonists and antagonists into this structure immediately after the sample phase.

2. Materials and methods

2.1. Animals

Adult (3-month-old, 300–330 g) and juvenile (22–30 postnatal days of age) male CRlCembe: Wistar rats were used, purchased from the Centro de Modelos Biologicos Experimentais (CeMBE) of this university. Animals were housed four to a cage under a 12/12-h light/dark cycle (lights on at 7:00 AM). All experiments were performed during the light phase of the cycle, at a constant temperature of 22 ± 1 °C and with free access to food and water. All procedures were in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Animal Committee on Ethics in the Care and Use of Laboratory Animals of the PUCRS. Throughout this work N was 10–12 animals per group.

2.2. Surgery and histology

The animals were anesthetized with intraperitoneal ketamine (75 mg/kg) plus xylazine (10 mg/kg), and then placed in a stereotaxic frame (David Kopf Instruments) and implanted bilaterally into the IC (IC; anterior, +1.2 mm; lateral, ± 5.5 mm; ventral, −3.1 mm; from bregma) according to coordinates from the 1986 atlas of Paxinos and Watson [39] with 22-gauge stainless steel guide cannulae. Acrylic cement was used to affix the guide cannulae to the skull. Animals were allowed to recover from surgery for at least 7 days. Before the behavioral experiments animals were handled for 3 min per day for 4 consecutive days. Cannulae placements were verified 2 days after the last behavioral procedure by infusing 4% methylene blue into the IC (0.5 μl/side). Spread of the dye was taken as an estimate of that of the drug infusions in the same animal [2,20,21,29,40]. As is usual in this laboratory [2,20,41] cannula placements were considered correct when the spread was ≤1 mm^3 from the intended infusion site. This occurred in 98% of the animals (data not shown). Only findings from these animals were analyzed.

2.3. Pharmacological treatments

Drug infusions were bilateral in all cases. Each animal was gently restrained by hand and a tight-fitting 30-gauge infusion cannula was inserted into the guide cannulae. The tip of the injection needle extended 1.0 mm beyond the tip of the cannula. The needle was connected to a Hamilton microsyringe by polyethylene tubing. The intra-IC infusions (0.5 μl per side in all cases) was carried out over 60 s and the infusion cannula was left in place for an additional 60 s to ensure complete delivery of the drugs. The drugs and the doses used were: the NMDA glutamate receptor coagonist, D-serine (D-Ser, 50.0 μg/side); the NMDA glutamate receptor antagonist, AP5 (5.0 μg/side); the D1/D5 dopamine receptor agonist, SKF38393 (SKF, 12.5 μg/side); the D1/D5 dopamine receptors antagonist, SCH33390 (SCH, 1.5 μg/side); the β-adrenoreceptor agonist, Isoproterenol (Iso, 3.0 μg/side); the β-adrenoreceptor antagonist, timolol (Tim, 1.0 μg/side); the agonist of the H2 histamine receptor, dimaprit (Dima, 2.3 μg/side); and the H2 blocker, ranitidine (Rani,17.5 μg/side); the 5-HT_{1A} serotonin receptors agonist, 8-OH-DPAT (6.25 μg/side); the 5-HT_{1A} serotonin receptors antagonist, NAN-190 (1.25 μg/side). The doses were chosen based on previous data in which their effects on memory in the same animal population were observed, often in dose-response curves [2,20,21,29,40]. All drugs were purchased from Sigma–Aldrich (St. Louis, MO, USA) and dissolved in sterile 0.9% saline. Control animals received equal volumes of infusion (0.5 μl per side into the IC) of drugs or saline (0.9% NaCl).

2.4. Social recognition memory (SRM) task

Social recognition memory was studied using the standard procedures used by Engelmann [42], Zinn [20] and their coworkers. The apparatus used was a transparent Plexiglas open-field arena (60 cm × 40 cm × 40 cm) placed in a dimly illuminated room. Two identical transparent Plexiglas cylindrical cages (9-cm diameter by 13-cm high) were placed inside the arena near to the corners. The cylinder cages had small holes (1 cm diameter spaced by 1 cm diameter) on the wall, allowing the passage of olfactory cues, while preventing the direct interaction between adults and juveniles (Fig. 1A). The arena and the cylinder cages were cleaned with 70% ethanol before and after each use. Adult animals were habituated to the experimental arena by allowing them to freely explore it during 20 min per day for 4 consecutive days. The empty cylinder cages were kept inside the arena during the habituation session. The sample phase took place 24 h after the last habituation session. The adults were individually placed in the center of the arena and allowed, for 1 h, to freely explore an unfamiliar juvenile placed in one of the cylinder cages and an empty cylinder. The retention test was 24 h later, the adults were placed in the same arena with the previously presented juvenile (familiar, F) and a second juvenile (novel, N) that had no prior contact with the adult placed in the cylinder that had been empty during the sample phase, and allowed to freely explore the set-up for 5 min. During the retention test, the exploration time spent by the adult on the F and the N juvenile was measured. Social exploratory behavior was defined as sniffing and touching the cylinder cages. The infusions into the IC were carried out immediately after the sample phase.

In order to quantify SRM, a discrimination index (DI) was calculated [43], DI = (time spent exploring N juvenile – time spent exploring the F juvenile)/total time spent exploring both juveniles. The DI may vary from 1 (exclusive exploration of the N juvenile) to −1 (exclusive exploration of the F juvenile). A positive DI indicates a preference for exploring the N juvenile, showing that the adult animal can recognize the F juvenile. The DI must be significantly different from zero, since a DI = 0 indicates that the adult animal has no preference for any