Behavioural Brain Research 313 (2016) 260–271

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

c-Fos expression predicts long-term social memory retrieval in mice

Thomaz Lüscher Dias[a,*], Hudson Fernandes Golino[b,c], Vinícius Elias Moura de Oliveira[a], Márcio Flávio Dutra Moraes[a], Grace Schenatto Pereira[a]

[a] Núcleo de Neurociências, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. Av. Pres. Antônio Carlos, 6627—Pampulha, Belo Horizonte, MG, Brazil
[b] Graduate School of Applied Computing, Universidade Estadual de Feira de Santana—Av. Transnordestina, S/N, Feira de Santana, BA, Brazil
[c] Graduate School of Psychology, Universidade Salgado de Oliveira. Rua Marechal Deodoro 263, Niterói, RJ, Brazil

HIGHLIGHTS

- Brain activity changes from acquisition to retrieval of a long-term social memory.
- Long-term social memory retrieval recruits the hippocampus and medial amygdala.
- Random Forest algorithm predicts long-term social memory retrieval.

ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form 26 June 2016
Accepted 18 July 2016
Available online 19 July 2016

Keywords:
Long-term social memory
c-Fos
Random Forest
Contextual integration
Top-down modulation

ABSTRACT

The way the rodent brain generally processes socially relevant information is rather well understood. How social information is stored into long-term social memory, however, is still under debate. Here, brain c-Fos expression was measured after adult mice were exposed to familiar or novel juveniles and expression was compared in several memory and socially relevant brain areas. Machine Learning algorithm Random Forest was then used to predict the social interaction category of adult mice based on c-Fos expression in these areas. Interaction with a familiar co-specific altered brain activation in the olfactory bulb, amygdala, hippocampus, lateral septum and medial prefrontal cortex. Remarkably, Random Forest was able to predict interaction with a familiar juvenile with 100% accuracy. Activity in the olfactory bulb, amygdala, hippocampus and the medial prefrontal cortex were crucial to this prediction. From our results, we suggest long-term social memory depends on initial social olfactory processing in the medial amygdala and its output connections synergistically with non-social contextual integration by the hippocampus and medial prefrontal cortex top-down modulation of primary olfactory structures.

1. Introduction

Multisensory stimuli have to be perceived as social in order to promote appropriate behavior during a co-specifics' encounter. At the same time, for social information to be further accessed, a social memory trace must be formed. The ability to identify and recognize individuals of their own species is one of the fundamental bases of social organization in rodents [1–4]. Individuality in rodents is conveyed mainly through scents and odors present in urine and bodily secretions [3,5,6]. Several brain structures are involved in the recognition of individual scents and in the formation and retrieval of memory traces acquired from unique sets of co-specific odors [3].

Mice and rats process social odor information rather similarly (reviewed by [3,7–9]): socially relevant molecules are actively inhaled during social interaction and are detected in the Main Olfactory Epithelium (MOE) or the Vomeronasal Organ (VNO), which send axons into the Main (MOB) and Accessory (AOB) Olfactory Bulbs, respectively. Downstream from the main olfactory system (MOS) follows the Anterior Olfactory Nucleus (AON) and the Piriform Cortex (PIR), which project indirectly, via the cortical nucleus of the Amygdala (CoA), to the medial Amygdala (MeA) and directly to the Entorhinal cortex (EC). The accessory olfactory system (AOS) projects directly to the MeA. The Bed Nucleus of the Stria Terminalis (BNST) and the medial Preoptic Area (MPOA) also receive direct and indirect input (via MeA) from the AOS and indirect input (via Mea) from the MOS. CoA and MeA also project into the

* Corresponding author at: Núcleo de Neurociências, Departamento de Fisiologia e Biofísica Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antôni Carlos, 6627, CEP 31270-901, Campus Pampulha Belo Horizonte, MG, Brazil.
E-mail address: luxeredias@gmail.com (T. Lüscher Dias).

http://dx.doi.org/10.1016/j.bbr.2016.07.030
0166-4328/

neuroendocrine hypothalamus (HY), notably the Paraventricular Nucleus (PVN), which sends modulating oxytocinergic back projections to the MeA and to several other brain structures [10]. The MeA also projects to the Lateral Septum (LS), which in turn projects to the hippocampus (HC) [11]. High-order cortical structures, namely the medial prefrontal cortex (mPFC; infra- and prelimbic areas – IL and PL) have also been reported to process social information and to be crucial for LTSM formation and retrieval.

Solid evidence has been produced implicating the MeA and other social brain structures (PIR, LS, MPOA, BST, CoA) in general processing of socially relevant stimuli and in short-term social memory (STSM) in both mice and rats [7,8,12–17]. Increasing evidence starts to emerge supporting the idea that MeA [17,18], HC [19–22] and mPFC [18] might be playing crucial roles in the ability of mice to sustain long-term social memories (LTSM). Moreover, in spite of the broad current knowledge on which brain structures receive and process social odor information individually [5,23–25], less attention has been paid to how this processing occurs as a network during acquisition and retrieval of LTSM. Simultaneous brain activity and behavioral measurements have been used recently as an attempt to functionally correlate these two variables in experimental animals [26–28] and humans [29]. Mostly, *in vivo* electrical neuronal activity is measured using in-brain microelectrodes while simultaneous behavioral observations are made. Later, computational modeling is performed to predict behavioral responses based solely on neuronal activity.

Approaches like these allow for live associations between activity in discrete cerebral areas and output behavioral response [26,28], but are usually costly and limited in the number of areas and amount of neurons monitored at the same time. Immunodetection of c-Fos expression, on the other hand, is a low cost, readily usable tool for detecting neuronal activity simultaneously in multiple brain areas [25,30].

Despite the broad use of c-Fos quantification as a way to determine differential neuronal activation in discrete brain areas following behavioral tasks (i.e. structure-to-structure linear comparisons), application of modern Machine Learning (ML) techniques (i.e. multivariate non-linear analyses) to predict behavior from brain-wide c-Fos expression has not been performed so far.

Here we analyze how brain areas previously reported as involved in social information processing differentially express c-Fos during acquisition and retrieval of LTSM in mice. We also forward the use of a ML technique, Random Forest (RF), as a method to predict interaction with novel or familiar social stimuli from c-Fos expression in the analyzed brain areas. By doing this, we expected to detect differences between acquisition and retrieval of LTSM and to propose mechanisms of how these memories are formed in the rodent brain.

2. Materials and methods

2.1. Behavioral task

Adult (8- to 12-weeks old) C57BL/6J and juvenile SWISS (25- to 30- days old) male mice were used. Mice were purchased from the Animal Facility of Universidade Federal de Minas Gerais and were housed in groups since weaning and through experimental procedures. Animals were kept in climate-controlled shelves under a 12 h light/dark cycle, 22 ± 2 °C temperature and 55 ± 10% relative humidity. Food and water were available *ad libitum*. Experiments were conducted during the light phase of the cycle. All procedures were approved by the Animal Use Ethics Committee of Universidade Federal de Minas Gerais (Protocol 44/2013).

Behavioral protocols were conducted in a 28cm × 17cm × 12 cm plastic box containing clean bedding. Juvenile SWISS mice were presented inside a pierced acrylic cylinder and social interaction lasted 5 min. The amount of time of direct contact between the adult's nose or whiskers and the juvenile's body was considered social investigation time (SIT). All animals were habituated to the box during 30 min. After habituation, a juvenile was placed inside the acrylic cylinder for both NOVEL and FAMILIAR groups. CONTROL mice were allowed empty apparatus exploration for 5 min. All animals were returned to their home cages and 1.5 h later, CONTROL and NOVEL animals were perfused. FAMILIAR animals were kept in their home cages for 24 h for the social memory trace to consolidate [31]. On the next day, FAMILIAR mice were re-exposed to the same juvenile they had interacted with the previous day and another 5 min exploration with SIT measurement was allowed. Animals were returned to their home cages for 1.5 h for c-Fos expression, after which they were sacrificed and had their brains harvested.

Successful LTSM retrieval by FAMILIAR animals was accessed by calculating the Recognition Index (RI: SIT on second day/SIT on first day + SIT time on second day). ARI of less than 0.5 is expected if animals explore the juvenile for a lesser amount of time on the second encounter, meaning they were able to remember the juvenile 24 h later. [22].

The reasons for the use of SWISS male juveniles as the source of social stimulation were twofold. First, we wanted to limit potential aggressive or sexual responses of our subjects, typically seen in adult male/male and male/female interactions. Presenting a juvenile allowed for evaluation of more purely social memory related responses [32,33]. Second, unpublished observations from our group have shown C57BL6/J adult-SWISS juvenile interaction leads to a greater reduction in second encounter SIT than interactions between two C57BL6/J animals. Due to its diverse genetic background [34,35], we argue SWISS mice produce more salient individual olfactory signatures, leading to easier discrimination.

In order to investigate brain activation pattern following novel or familiar social interaction, we performed anti-c-Fos immunohistochemistry immediately after social investigation. c-Fos is highly expressed from 1 to 2 h after neuronal activation [36]. Immunoquantification of this protein is a reliable way to determine behaviorally elicited neuronal activation from immediately before the 1–2 h period [37]. C57BL/6 J mice (n = 23) were divided in three groups (n = 7–8 per group): CONTROL, NOVEL and FAMILIAR. All groups were submitted to variations of the Social Recognition Task [4,22] in the above described apparatus. Fig. 1 illustrates this experimental design.

2.2. Sampling brain structures of interest

In order to be able to analyze a significant amount of functional circuits possibly involved in LTSM acquisition and retrieval, we decided to sample key brain structures related to (1) primary social odor processing, (2) social/sexual/aggressive behavior, (3) emotional/contextual memory formation and (4) high-order cortical processing [3,30]. The structures chosen were the following for each functional category: (1) MOB glomerular (GL), mitral (MI) and granule cell (GR) layers, AOB granule cell layer (AOB), piriform cortex (PIR); (2) LS dorsal layer (LSD), LS ventral layer (LSV), hypothalamic lateral area (LH), hypothalamic paraventricular nucleus (PVN); (3) hippocampal dentate gyrus (DG), hippocampal CA3 area (CA3), hippocampal CA2 area (CA2), hippocampal CA1 area (CA1), basolateral amygdala (BLA), posterodorsal portion of MeA (MeApd), (4) pre-limbic cortex (PL) and infra-limbic cortex (IL).

2.3. Anti-c-Fos immunohistochemistry

After 1.5 h, animals were deeply anaesthetized with Xilazine (10 mg/kg) and Ketamine (100 mg/kg) and transcardially perfused