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Recognition memory-induced gene expression in the perirhinal cortex: A transcriptomic analysis

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ABSTRACT

We have used transcriptome analysis to identify genes and pathways that are activated during recognition memory formation in the perirhinal cortex. Rats were exposed to objects either repeatedly, so that the objects become familiar, or to novel objects in a bow-tie maze over six consecutive days. On the final day, one hour after the last exposure to the series of objects, RNA from the perirhinal cortex was sequenced to compare the transcriptome of naïve control rats and rats exposed to either novel or familiar stimuli. Differentially expressed genes were identified between group Novel and group Familiar rats. These included genes coding for transcription factors, GDNF receptors and extracellular matrix-related proteins. Moreover, differences in alternative splicing were also detected between the two groups, which suggests that this post-transcriptional mechanism may play a role in the consolidation of object recognition memory. To conclude, this study shows that RNA sequencing can be used as a tool to identify differences in gene expression in behaving animals undergoing the same task but encountering different exposures.

1. Introduction

Recognition memory allows us to make judgements regarding whether we have encountered something before, and hence to discriminate a novel from a familiar stimulus. A number of studies in both humans and animals have shown that such familiarity discrimination depends on the integrity of the perirhinal cortex in the medial temporal lobe [1–3]. It has been shown that protein synthesis in the perirhinal cortex is necessary for the consolidation of long-term recognition memory [4]. Furthermore, several immediate early genes, e.g. *c-Fos* and transcription factors, which are increased during recognition memory are suggested to have a causal role [5–11]. For example, a repeated observation is an increase in the number of c-Fos positive neurons in the perirhinal cortex in response to novelty [5,11,12,13]. Furthermore, levels of the transcription factor cAMP response element-binding protein (CREB), itself an activator of immediate early genes, were found to be increased in the perirhinal cortex of rats exposed to novel compared to familiar objects [10]. Other immediate early genes functionally associated with recognition memory include early growth response 1 (Egr1; also known as Zif268) [7], activity-regulated cytoskeleton-associated protein (Arc) [6] and nuclear receptor 4a family member NR4a2 [8]. The observation that transcription factors are required in recognition memory suggests that gene expression may be an important process underlying long-term recognition memory formation.

To better understand the changes in gene expression that forge recognition memory, we have profiled the transcriptome of the perirhinal cortex following a recognition memory task. Using RNA sequencing we aimed to investigate the differences in gene expression between rats that encountered novel objects and those that saw highly familiar objects. The animals were exposed to the objects using a bow-tie maze [14], which allows recognition to be tested over multiple trials, and previous studies have shown that this protocol results in differential *c-Fos* expression selectively in the caudal perirhinal cortex, in response to exploration of novel and familiar objects [14].

In addition to the exploratory nature of this study, we aimed to investigate specific gene groups and pathways that have been associated with recognition memory. Firstly, due to the previously observed association of transcription factors with exposure to novelty, we predicted an increase in the expression of transcription factors and immediate early genes in the perirhinal cortex of group Novel compared to Control or Familiar. Secondly, signalling pathways such

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as the neurotrophic signalling pathway have been shown to be important in the first two hours following exposure to novel objects [15–18]. Therefore we hypothesised that an upregulation of transcripts coding for neurotrophic factors and receptors might occur in group Novel. Thirdly, we explored the potential role of alternative splicing in the perirhinal cortex.

2. Materials and methods

2.1. Animals

Male Lister Hooded rats (~350–450 g; Harlan Laboratories, UK) were used for all experiments. Rats were kept on a reversed 12-h light/dark cycle (lights on 20.00-08.00 h) and all behavioural testing took place during the dark phase. Prior to the start of the behavioural experiments, the animals were placed on food restriction with daily access to food for a period of 2 h. The rats were kept at over 85% of their free-feeding weight. Water was available without restriction.

All animal procedures were conducted in accordance with the United Kingdom Animals Scientific Procedures Act (1986) and associated guidelines. All efforts were made to minimise any suffering and the number of animals used.

2.2. Behavioural task

The bow-tie maze task [14] consisted of two phases – pre-training and training – followed by the test session (Fig. 1a). The rats were divided into three groups – group Familiar, group Novel and group Control. Rats were housed so that one rat from group Familiar and one from group Novel shared a cage to ensure the rats were treated similarly across groups. Control rats were also food restricted and handled daily throughout the procedure but they were not subjected to any behavioural training or testing.

2.2.1. Apparatus

Testing took place in a bow-tie shaped maze consisting of a grey wooden floor and metal walls. A guillotine door that could be operated manually separated the two triangular sides of the maze. At each end of the maze a food well was attached centrally to the floor, which could be baited with a food reward in the form of a sucrose pellet (OmniTreat™ 45 mg tablets; TestDiet, Sandown Scientific, Hampton, UK). Objects were placed into the left and right corners at either end of the maze. This set-up ensured that neither of the objects was associated with the food reward. The objects were junk objects, including toys, household items and decorative objects, in varied colours, shapes, materials and sizes. In between behavioural sessions, the objects were cleaned with 100% ethanol to eliminate olfactory cues. The animals' behaviour was recorded via an overhead camera.

2.2.2. Pre-training

In order for the rats to learn to shuttle from one end of the maze to the other when the sliding door was opened and to collect a food reward, the animals were subjected to daily training for 7 days. On day 1 the rats were allowed to explore the arena (without the guillotine door in place) in pairs for 30 min. Sucrose pellets were distributed across the maze and in the food wells. On days 2 and 3, the rats were allowed to explore the maze individually for 20 min. Only the food wells contained reward pellets (one pellet at a time) and they were constantly re-baited to encourage shuttling from one side of the arena to the other. Days 4 and 5 were the same except that the central guillotine door was used to control the movement of the rat. On days 6 and 7 three different pairs of objects were introduced. These were not used in the training phase.

2.2.3. Training

The training phase consisted of two training sessions per day, one in the morning and one in the afternoon, over 6 consecutive days (training sessions 1–12). The set-up of the training sessions is displayed in Fig. 1b. At the start of each training session the rat, from group Novel or group Familiar, was placed into one side of the arena with the guillotine door shut, where it encountered one object A which it had the opportunity to explore for 1 min. The guillotine door was raised and the rat then moved across to the other side where it encountered an identical copy of object A and a different object B (Trial 1). After 1 min the sliding door was raised again to enable the rat to shuttle across where it found an identical copy of object B and object C (Trial 2). This continued for 20 trials using a total of 21 object pairs. In each trial the rat was allowed to collect one sucrose pellet from the food well. The position of the familiar object in relation to its previously viewed copy (same side, opposite side) was counterbalanced across each session. Furthermore, the order in which the objects were shown (either A to U or U to A) was counterbalanced across each rat group for each session.

Group Familiar saw the same 21 objects during every training session in a different order so that by the end of the training period the rats should be highly familiarised to this set of 21 objects. In the test session again the animals were presented with copies of the highly familiarised objects. Twenty-one different objects were used to match the sensory demands of the task.

The rats in group Novel received object exposure in the same way, i.e. rats were allowed to explore one novel object and one familiar object (familiar because it had been encountered at the other end of the maze in the preceding trial). For the first 6 training sessions, 21 different novel objects were used in every session. For the last 6 training sessions, these 126 objects were re-used but grouped into different sets of 21 objects and shown in a different order. In the test session group Novel saw the same 21 objects as group Familiar, hence all the conditions (objects seen, motor demands, length of exposure to the objects) were identical between the groups. As before, each test trial for group Novel comprised one novel object and one object encountered for a maximum of one minute in the previous trial.

On the last three training sessions, all rats were individually placed into a holding cage after each run for approximately 1 h to habituate them to this process for the test day. Group Control was habituated in the same way.

2.2.4. Perirhinal cortex extraction

In the test session the rats were run on the task as described and then placed in a holding cage in a quiet and darkened room to minimise exposure to extraneous stimuli. One h after the end of the session rats were placed into an anaesthetic induction box filled with isoflurane until the heart stopped and guillotined. The brains were extracted, immediately frozen on dry ice and stored at −80 °C prior to dissection of the perirhinal cortex and RNA extraction. For group Control the same protocol was followed but without the prior behavioural test.

2.2.5. Statistical analysis

Exploration of an object was defined as the rat directing its nose towards the object at a distance of < 1 cm from the object. Sitting on the object or looking up while resting against the object was not counted as exploration. As a measure of behavioural performance, a discrimination ratio was calculated from the recorded object exploration (E) using the following formula:

$$Discrimination\ ratio = \frac{E_{novel} - E_{familiar}}{E_{total}}$$

For group Familiar (except for the first session), the E_{novel} and $E_{familiar}$ refer to the less recently seen familiar object (last seen in the previous session) and the more recently seen familiar object (examined on the previous trial), respectively. The discrimination ratio was recalculated after every trial of a session (taking into account the exploration of all preceding trials of that session) yielding the updated discrimination ratio. Repeated-measures ANOVA was used to draw