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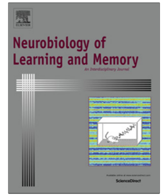

Cellular activation of hypothalamic hypocretin/orexin neurons facilitates short-term spatial memory in mice

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ABSTRACT

The hypothalamic hypocretin/orexin (HO) system holds a central role in the regulation of several physiological functions critical for food-seeking behavior including mnemonic processes for effective foraging behavior. It is unclear however whether physiological increases in HO neuronal activity can support such processes. Using a designer rM3Ds receptor activation approach increasing HO neuronal activity resulted in improved short-term memory for novel locations. When tested on a non-spatial novelty object recognition task no significant difference was detected between groups indicating that hypothalamic HO neuronal activation can selectively facilitate short-term spatial memory for potentially supporting memory for locations during active exploration.

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1. Introduction

Foraging requires the coordination of higher-order neural systems that lead to a heightened sensory awareness, effective decision-making, and mnemonic functions for remembering objects and locations previously experienced. The engagement of these circuits must ultimately be derived from primary brain circuits that respond to energy deficiencies for increasing arousal for engaging food seeking behavior and achieving energy homeostasis. The hypocretin/orexin (HO) system holds a central role in the regulation of activity for exploration as it has been shown to be a key system in promoting food intake, arousal, reward, and, importantly, food anticipatory activity (Carter, Adamantidis, Ohtsu, Deisseroth, & de Lecea, 2009; De Lecea, 2010; Kotz, 2006; Sakurai et al., 1998; Zhang, Yu, Zhuang, Zhu, & Wang, 2013). Although the cell bodies of HO neurons are confined to the lateral hypothalamus (LH), they project extensively to the entire neuroaxis (Tsunematsu & Yamanaka, 2012) supporting a role for this system in coordinating multiple brain systems for effective foraging behavior, including extra-hypothalamic memory-related regions (Nambu et al., 1999; Peyron et al., 1998). In agreement with the above neuroanatomy, pharmaco-behavioral experiments have demonstrated a functional role for the HO system in hippocampal and amygdala-dependent memory processing (Jaeger, Farr, Banks, & Morley, 2002; Palotai, Telegdy, Ekwerikea, & Jászberényi, 2014; Sears et al., 2013; Telegdy & Adamik, 2002). Electrophysiology studies however have revealed that HO neurons can functionally excite and inhibit neurons independent of HO release/receptor activation (Apergis-Schoute et al., 2015; Schöne, Apergis-Schoute, Sakurai, Adamantidis, & Burdakov, 2014). Despite these studies linking HO receptor activation to mnemonic functions it is unclear whether physiological increases in HO activity alone can support such processes.

The aim of this study was to test the effects of HO neuronal activation on short-term memory for locations and objects. To do so, a genetic tool – the designer receptors exclusively activated by designer drugs (DREADDs) – was implemented in an HO-cre$^+$ transgenic mouse line in order to directly excite HO neurons during task engagement. Due to potential confounding effects of food reward as a positive reinforcer the memory tasks in the present study were ones that were "unbaited", (without food), namely the spontaneously alternating T-maze (Deacon & Rawlins, 2006; Lalonde, 2002) and the spontaneous object recognition task (Bussey, Muir, & Aggleton, 1999; Leger et al., 2013; Winters & Bussey, 2005) both of which are thought to be motivated by the instinct to explore novel locations and objects.

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2. Materials and Methods

2.1. Animal subjects

All animal procedures were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986. Male HO-cre$^+$ mice were bred in heterozygous: wild-type (wt) breeding pairs with C57BL/6J mice and were genotyped using PCR from ear notch biopsy. Heterozygous HO-cre$^+$ and their wt littermates (Matsuki et al., 2009; Sasaki et al., 2011) were selected and used for all the behavioral tests after the age of 9 weeks. The animals were group housed (2–4 animals per cage), under temperature- and humidity-control conditions, on a 12-h light/dark reversed cycle (7 a.m. lights off). Water and food were available ad libitum during all the experimental procedures.

2.2. Stereotaxic operations and viral gene transfer

A Cre-inducible AAV_5-hSyn-DIO-rM3Ds-mCherry or AAV_5-hSyn-DIO-mCherry viral construct (titer $4 * 10^{12}$ genome copies/mL, University of North Caroline, Gene Therapy Center, NC, USA) was bilaterally injected into the LH of male HO-cre$^+$ and wt littermate mice. Ten-week old HO-cre$^+$ mice and their wt littermates were anesthetized with a mixture of isoflurane (5% induction, 1–2% maintenance) and oxygen (flow rate 0.8–1.0 L/min), and placed into a stereotactic frame. A small bilateral craniotomy was performed for injections of viral constructs (150 nL) at 0.1 μL/min (antero-posterior −1.35 mm from bregma, laterally ±0.95 mm from midline and −5.4 mm, −5.2 mm and −5.0 mm from the skull surface). Postoperatively, mice received meloxicam (1 mg/kg. s.c., Boehringer Ingelheim Ltd. Bracknell, UK) and behavioral tests started after a 5-week period to allow for sufficient protein expression. All surgeries were done using aseptic surgical techniques.

2.3. Electrophysiology

Coronal slices were made >9 weeks post-injection and recordings were made as in our previous study (Apergis-Schoute et al., 2015). Data were analyzed with Minianalysis (Synaptosoft), Igor and Adobe Illustrator/Photoshop. Whole-cell recordings were performed at 35 °C using an EPC-10 amplifier and Patch-Master software (HEKA Elektronik).

2.4. Chemicals and solutions

ACSF was gassed with 95% O_2 and 5% CO_2, and contained the following (mM): NaCl 125, $NaHCO_3$ 25, KCl 3, NaH_2PO_4 1.25, $CaCl_2$ 1/2 (cutting/recording), $MgCl_2$ 6/1 (cutting/recording), sodium pyruvate 3 and glucose 25/5 (cutting/recording). Pipettes were filled with (in mM): potassium gluconate 135, NaCl 7, Hepes 10, Na_2-ATP 2, Na-GTP 0.3, and $MgCl_2$ 2; pH was adjusted to 7.3 with KOH. Clozapine-N-oxide (CNO, Sequoia Research Products Ltd, Pangbourne, UK) was freshly dissolved in sterile saline (0.9% NaCl) with 0.5% DMSO and administered by intraperitoneal injection (i.p.) 30 min prior to behavioral tasks. All chemicals were from Sigma, Tocris, or Bachem.

2.5. Transcardial perfusion and tissue preparation

Mice were anaesthetized with 500 mg/kg of sodium pentobarbital and were tested for absence of paw pinch response. Mice were perfused with 50 mL of phosphate buffer solution (PBS) and subsequently with 50 mL of 4% paraformaldehyde at pH 7.4. Brains were removed and post-fixed in 4% paraformaldehyde overnight and then kept in 30% sucrose for cryoprotection. Coronal sections of 30 μm were cut by a sliding microtome.

2.6. Immunohistochemistry

The primary and secondary antibodies used were as follows: primary; goat anti-c-Fos (1:800, Santa Cruz Biotechnology, sc-52-G, Santa Cruz, CA, USA) and rabbit anti-orx-A (1:1000, Phoenix Pharmaceuticals, H-003-30, Burlingame, CA, USA). Secondary; donkey Biotin-SP-conjugated anti-goat (1:500, Jackson ImmunoResearch, 705-065-003, Jennersville, PA, USA) and AlexaFluor 488-conjugated goat anti-rabbit (1:1000, Abcam, ab150077, Cambridge, UK). All antibodies were diluted in blocking solution (0.3% TritonX-100, 10% donkey serum, 1% BSA in PBS). Sections were incubated at room temperature and rinsed 3 × 5 min in PBS, then incubated for 1 h in 0.3% H_2O_2 in PBS and subsequently in blocking solution, for 1 h and incubated in anti-c-Fos primary antibody, overnight. The next day sections were incubated in biotinylated anti-goat secondary antibody for 1 h, followed by 5-min incubation in peroxidase-conjugated avidin/biotin complex, ABC solution (Vector laboratories, Peterborough, UK) and in DAB substrate. Sections were then washed, mounted onto slides and cover-slipped. Images were captured with a Zeiss fluorescence microscope and analyzed by ImageJ software.

2.7. Behavioral assays

2.7.1. Open field test

An open field test was used to assess potential differences in locomotor activity and novelty-induced anxiety levels between the groups (Hall & Ballachey, 1932). Mice were injected with CNO (1 mg/kg, i.p.) and 30 min later were allowed to move freely into an cylindrical open field for a total test time of 30 min, under low light intensity (<50 lx) conditions. The arena of 36 cm in diameter was used to test mouse locomotor activity and anxiety-like behavior in an open field and was divided into three 12-cm wide circular zones (outer, middle, and center zone) (Fig. 2A). Video recording was used to track the open field behavior, by measuring the total distance moved and time spent in each zone. Subsequently, off-line video analysis (EthoVision version 11, Noldus Information Technology, Wageningen, The Netherlands) was conducted to quantify behavior.

2.7.2. Spontaneous alternation T-maze test

Spontaneous alternation in a T-maze was used to assess working memory abilities, based on the innate tendency of mice (and rats) to alternate their choice of a novel goal arm based on the recall of their initial choice (Olton & Papas, 1979). The T-maze used was made of black painted wood and consisted of one central arm (starting area) and two goal-arms, with dimensions 30 cm × 10 cm each and walls 20 cm in height, as well as a removable central divider extending 5 cm into the start arm. The protocol used was based on the work of Deacon and Rawlins (2006). Vehicle or CNO (1 mg/kg, i.p.) was administered in a pseudo-randomized way, 30 min prior to the test, and mice were placed in the start arm with the central divider in place so that when entering either of the goal arms the mice were not able to see the other one. After the mice entered either of the arms, a black painted wooden wall placed in front of the chosen arm's entrance, to allow them to explore the arm for 30 s. After a delay of 0 or 5 min, mice were returned to the start arm, facing away from the goal arms, with the central divider and wall removed. A schematic representation of the T-maze apparatus and the behavioral assay used is provided in Fig. 3A. Mice were tested once daily at a constant time and injected with CNO or vehicle on alternate days. Trials on which the mice did

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