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Research report

Involvement of serotonin 2A receptor activation in modulating medial prefrontal cortex and amygdala neuronal activation during novelty-exposure

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HIGHLIGHTS

- Ketanserin blocks novelty-induced neuronal activation in medial prefrontal cortex.
- This is accompanied by decreased neuronal activation in the basolateral amygdala.
- Ketanserin makes basolateral amygdala more reactive towards the anxiogenic stimulus.
- 5-HT2A blockade does not affect activation of striatal projecting amygdala neurons.

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ABSTRACT

The medial prefrontal cortex (PFC) plays a major role in executive function by exerting a top-down control onto subcortical areas. Novelty-induced frontal cortex activation is 5-HT_{2A} receptor (5-HT_{2A}R) dependent. Here, we further investigated how blockade of 5-HT_{2A}Rs in mice exposed to a novel open-field arena affects medial PFC activation and basolateral amygdala (BLA) reactivity. We used c-Fos immunoreactivity (IR) as a marker of neuronal activation and stereological quantification for obtaining the total number of c-Fos-IR neurons as a measure of regional activation. We further examined the impact of 5-HT_{2A}R blockade on the striatal-projecting BLA neurons. Systemic administration of ketanserin (0.5 mg/kg) prior to novel open-field exposure resulted in reduced total numbers of c-Fos-IR cells in dorsomedial PFC areas and the BLA. Moreover, there was a positive correlation between the relative time spent in the centre of the open-field and BLA c-Fos-IR in the ketanserin-treated animals. Unilateral medial PFC lesions blocked this effect, ascertaining an involvement of this frontal cortex area. On the other hand, medial PFC lesioning exacerbated the more anxiogenic-like behaviour of the ketanserin-treated animals, upholding its involvement in modulating averseness. Ketanserin did not affect the number of activated striatal-projecting BLA neurons (measured by number of Cholera Toxin b (CTb) retrograde labelled neurons also being c-Fos-IR) following CTb injection in the ventral striatum. These results support a role of 5-HT_{2A}R activation in modulating mPFC and BLA activation during exposure to a novel environment, which may be interrelated. Conversely, 5-HT_{2A}R blockade does not seem to affect the amygdala-striatal projection.

1. Introduction

The serotonin 2A receptor (5-HT_{2A}R) has received considerable interest as an important potential target in the treatment of neuropsychiatric disorders such as schizophrenia, obsessive compulsive disorder and borderline personality disorder [1–4]. The 5-HT_{2A}R is highly expressed in the prefrontal cortex (PFC) [5] and exerts an important role in modulating PFC activity and neural oscillations [6–11].

The PFC is a key structure for executive functions [12,13], including cognitive processes such as reasoning, judgment and value-based decision-making, by integrating inputs from multiple brain regions and exerting top-down control on these regions

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in a coordinated manner [14,15]. Exposure to a novel environment engages this circuitry [16–18] with the medial PFC (mPFC) playing a central role in the processing and risk-evaluation of novelty, as seen in humans [19] and rodents [20,21]. Emotional valuation is integrated in the decision-making circuitry through the mPFC (risk assessment and inhibitory control), the basolateral amygdala (BLA) (emotional learning) and the ventral striatum (VS) (integrating information about reward, context and motivational drive); all parts of an evaluation system [22–25] where excitatory transmission from BLA to VS facilitates reward-seeking behaviour [26,27]. Further, there is a functional dissociation across mPFC subregions, with activation of the dorsal parts being related to the risk/uncertainty and the ventral parts with the subjective gain/loss valuation associated with a decision [28].

Rodent studies have reported c-Fos immunoreactivity (IR) and mRNA levels to be increased in the PFC after exposure to novelty [29–33]. C-Fos is an immediate-early gene widely used as a marker of general neuronal activity [34,35]. The rapid increase in immediate-early gene expression acts as first-response proteins [35,36], leading to a number of events involved in long-term functional adaptations of the PFC such as synaptic plasticity and memory consolidation [37]. The strongest c-Fos induction occurs during the early phases of behavioural training, when a learning process is commonly taking place integrating memory-dependent processes in the brain [31,33,38–40].

We have previously reported that novelty-induced *c-fos* expression in the PFC is blocked by systemic ketanserin administration and is 5-HT_{2A}R, not 5-HT_{2C} receptor, dependent [29]. This suggests a crucial role for this receptor in the PFC-mediated top-down control of risk-assessment and processing of reward-seeking behaviour during a novelty-exposure. As shown by functional imaging studies there is an inverse relationship between density of PFC 5-HT_{2A}Rs and amygdala reactivity in humans exposed to fearful stimuli [41]. Further, ketanserin in humans leads to more risk-aversive behaviour [42]. The aim of the present study was to explore the role of the different subregions of the mPFC and 5-HT_{2A}R activation in relation to amygdala reactivity during novelty-exposure. Also we wanted to investigate whether the 5-HT_{2A}R had a modulatory role on the activation of BLA neurons projecting to the VS.

Mice receiving systemic injections of ketanserin or vehicle were exposed for five minutes to an open-field arena in order to induce mPFC activation as done previously [29]. Further, the time spent in border versus centre was monitored during the five minutes exposure. A well-validated and strongly reliable stereological counting method [43,44] was applied for the quantification of total c-Fos-IR neurons in the mPFC, differentiating between the dorsomedial (dmPFC) and ventromedial (vmPFC) PFC, and in the BLA. In order to establish the involvement of the mPFC, animals received a priori a unilateral NMDA or sham lesion into the mPFC. Moreover, all animals were injected one week before the novelty assay with a retrograde tracer (Cholera toxin subunit b (CTb)) into the VS, enabling us to identify and quantify the fraction of c-Fos expressing BLA neurons projecting to the VS.

2. Experimental procedures

2.1. Animals

The animal care and experimental procedures were performed in accordance with the European Community Councils Directive of Nov 24th 1986 (86/609/EEC) by The Danish National Committee for Ethics in Animal Research under the Danish Ministry of Justice (License number 2010/561-1834). Female C57Bl mice (N = 48), around 12 weeks-old (21–25 g), were group-housed (eight in each cage) and kept in a controlled environment with a 12/12 h light/dark cycle, provided with standard rodent diet and water *ad libitum*. The animals were allowed to acclimatise in the animal facility for at least one week prior to the day of the first surgery and they were randomly assigned to experimental groups.

2.2. Surgery

First, we performed excitotoxic/sham lesions. After one week, retrograde tracing was performed on all mice. At the time of surgery, the mice were anesthetized with a solution of ketamine (2.1 mg/ml) and dexmedetomidine (0.016 mg/ml) (20 ml/kg, i.p.). The mice were placed in a stereotaxic frame (Stoelting, Wood Dale, IL), the skull was exposed and a hole was drilled for the unilateral intracerebral injections. For the excitotoxic lesions, 0.5 ul of NMDA (22 mg/ml; Sigma, St. Louis, MO) dissolved in sterile 0.1 M phosphate-buffer (PB) (pH 7.4) or vehicle (sterile 0.1 M PB, pH7.4) were injected into the mPFC at the following coordinates: +2.9 mm anterior to Bregma; −0.25 mm lateral to the midline; and dorsoventral −2.0 mm below the dura. For the retrograde tracing, 0.2 ul 0.4% CTb (# 104, List Biological Laboratories, Campbell, CA, USA) were injected at the following coordinates for the VS: + 2.2 mm anterior to Bregma; −0.75 mm lateral to the midline; and dorsoventral −4.25 mm below the dura. All coordinates were referenced to Bregma according to a standard Paxinos mouse brain stereotaxic atlas and further adjusted in pilot studies. The drugs were delivered with a 5 μl Hamilton syringe (75 RN; 34s/15/3; Hamilton, Bonaduz, Switzerland) slowly through 3 min and the syringe was left in place for another 5 min in order to prevent spreading of the drug along the needle track. All animals were given 1 ml of saline solution i.p. post-surgery and returned to cage placed on a heating element (28°) to aid recovery. Just before wake-up, meloxicam (20 ml/kg, s.c.) was administered for analgesic effect.

2.3. Novelty paradigm and locomotor activity assessment

One week after retrograde tracing, all mice were exposed to a novelty paradigm. All mice were handled for three days prior to the open-field test by the experimenter. The mice were acclimatized to the experimental room overnight. At the time of open-field testing, the mice were injected with ketanserin tartrate (0.5 mg/kg; 20 ml/kg, i.p.) (Sigma-Aldrich, St Louis, MO, USA) or its vehicle (sterile distilled water (20 ml/kg, i.p.)) and returned to their home-cage. Each treatment group was placed in separate cages to prevent the response of one group to influence the response of another. After 30 min the mice were placed in the middle of the open-field arena (35 × 35 × 40 cm) situated in a dimly lit room. After 5 min the novelty-exposed mice were returned to their respective home-cage for another 30 min before receiving an overdose of ketamine/dexmedetomidine solution and transported to a separate room for blood collection and perfusion fixation of the brains. As a control for basal versus open-field induced c-Fos-IR levels four mice were held in a home-cage in the same room while the open-field testing took place and handled shortly at a similar time point as the open-field group, but without being exposed to the open-field.

Behavioural activity during the open-field exposure was recorded by a webcamera located on the ceiling above the open-field arena. The total distance moved by the animals and the total time the animal spent in the centre of the arena (31.5 × 31.5 cm) versus the border (3.5 cm) was analysed and calculated with the video-tracking software EthoVision (version XT 9, Noldus, Wageningen, the Netherlands).