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NOVELTY-INDUCED ACTIVITY-REGULATED CYTOSKELETAL-ASSOCIATED PROTEIN (*ARC*) EXPRESSION IN FRONTAL CORTEX REQUIRES SEROTONIN 2A RECEPTOR ACTIVATION

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Abstract—Many psychiatric disorders are characterized by cognitive and emotional alterations that are related to abnormal function of the frontal cortex (FC). FC is involved in working memory and decision making and is activated following exposure to a novel environment. The serotonin 2A receptor (5-HT_{2A}R) is highly expressed in the FC where its activation induces hallucinations, while blockade of 5-HT_{2A}Rs contributes to the therapeutic effects of atypical antipsychotic drugs. The purpose of the present study was to investigate the involvement of 5-HT_{2A}R in FC activation following exposure to a novel environment. As an output of FC activation we measured expression of activity-regulated cytoskeletal-associated protein (*Arc*). Novelty-exposure (open-field arena) robustly up-regulated FC *Arc* mRNA expression (~160%) in mice compared to home-cage controls. This response was inhibited with the 5-HT_{2A}R antagonists ketanserin and MDL100907, but not with the selective 5-HT_{2C}R antagonist SB242084. Novelty-exposure also induced *Arc* mRNA expression in hippocampus (~150%), but not in cerebellum or brainstem. Pretreatment with 5-HT_{2A}R antagonist ketanserin did not repress the *Arc* induction in hippocampus, indicating that the involvement of 5-HT_{2A}R in this response is restricted to the FC. Similarly, the novelty-induced stress as determined by increasing levels of plasma corticosterone, was not influenced by 5-HT_{2A}R antagonism suggesting that *Arc* mRNA and stress are activated via distinct mechanisms. Taken together, our results demonstrate that the induction of *Arc* in the FC following exposure to a novel environment is dependent on the 5-HT_{2A}R, and that the simultaneous release of corticosterone is regulated via another system independent of 5-HT_{2A}R activation.

Key words: 5-HT_{2A}, serotonin, novelty, IEG, c-fos, mouse.

The serotonin 2A receptor (5-HT_{2A}R) is highly expressed in the frontal cortex (FC) (Cornea-Hebert et al., 1999), a crucial brain region involved in working memory, attention and decision making (Robbins, 2000). Recent studies have revealed that as in hippocampus FC networks can undergo dynamic neuronal adaptation processes through the induction of synaptic plasticity. The actions of 5-HT_{2A}R signaling in FC are at least partly mediated through release of glutamate (Beique et al., 2007). This excitatory activity can in turn lead to increases in the expression of immediate-early genes (IEGs), which act as the earliest genomic response to synaptic activity (Clayton, 2000). The cascade of events initiated by rapid induction of these gene products are important for the long-term functions of FC such as synaptic plasticity (Goto et al., 2010), and compromised induction of synaptic plasticity in the FC is seen in schizophrenia (Goto et al., 2010). These studies support that 5-HT_{2A}R signaling is important for FC mediated behaviour and that this receptor is an important pharmacological target for the treatment of neuropsychiatric disorders.

Activity-regulated cytoskeletal-associated protein (*Arc*) is a brain-specific effector IEG product that plays a key role in the activity-dependent synaptic modifications underlying memory consolidation (Steward and Worley, 2002). Because of its engagement in structural synaptic plasticity, *Arc* is suitable for investigation of neuronal networks that underlie information processing (Bramham et al., 2008). *Arc* induction in response to physiological stimuli takes place in several cortical and subcortical areas in the rodent brain and shows a more region-specific pattern of expression than *c-fos*, a widely used IEG for brain activation mapping (Mikkelsen and Larsen, 2006; Ons et al., 2004, 2010).

Exposure to a novel environment induces *Arc* expression in a number of cortical subregions including the medial prefrontal cortex (mPFC), parietal cortex and the hippocampus (Klebaur et al., 2002; Vazdarjanova et al., 2002). Under the exposure to the open field, the FC is engaged in the evaluation of the stressfulness of the situation (Robbins, 2000). Thus, the exposure to novelty initiates a number of events that integrates risk assessment behaviour where both rewarding and aversive outcomes are possible. Accordingly, both the FC and the hypothalamo-pituitary adrenocortical (HPA) axis will be activated. Exposure to a novel environment activates 5-HT neurons in a subset of the dorsal raphe nucleus and increases extracellular 5-HT concentrations in the FC (Adell et al., 1997; Lowry et al., 2005). These data support the hypothesis that exposure to a novel environment requiring active exploration releases 5-HT which in turn induces *Arc* expression in the FC. However, it is not known which 5-HT receptor in the FC mediate the changes in IEG expression, but the 5-HT_{2A}R was considered to be an attractive candidate due to its role in FC neuronal control. The aim of this

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Abbreviations: ACTH, adrenocorticotropic hormone; *Arc*, activity-regulated cytoskeletal-associated protein; BDNF, brain-derived neurotrophic factor; CREB, cyclic AMP response element binding protein; Egr-2, early growth response gene-2; FC, frontal cortex; HPA, hypothalamic-pituitary-adrenal axis; IEG, immediate-early genes; LSD, lysergic acid diethylamide; mPFC, medial prefrontal cortex; PFC, prefrontal cortex; 5-HT_{2A}R, serotonin 2A receptor.

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study was therefore to characterize to what extent 5-HT_{2A}Rs are involved in FC activation induced by exploration of a novel environment.

EXPERIMENTAL PROCEDURES

Animals

All animal experiments were carried out in accordance with the regulations provided by the Danish Animal Experimentation Inspectorate under the Ministry of Justice. Eight weeks-old male B6D2F1 mice (Charles River Laboratories, Sulzfeld, Germany) were group housed (six 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 acclimatize in the animal facility for at least 5 days prior to the day of the experiment.

Drugs

Ketanserin tartrate [3-{2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl}quinazoline-2,4(1*H*,3*H*)-dione] (Sigma-Aldrich, St Louis, MO, USA), MDL100907 [(R)-(+)-alpha-(2,3-dimethoxyphenyl)-1-[2-(4-fluoro-phenyl)ethyl]-4-piperidinemethanol] (ABX, Radeberg, Germany) and SB242084 hydrochloride [6-chloro-5-methyl-*N*-{6-[(2-methylpyridin-3-yl)oxy]pyridin-3-yl]indoline-1-carboxamide] (Tocris Ltd, Bristol, UK) were dissolved 1:20 in dimethyl sulfoxide and 0.9% saline. DOI (Sigma-Aldrich) hydrochloride [(±)-2,5-dimethoxy-4-iodoamphetamine] was dissolved in 0.9% saline.

Experimental paradigm

Animals were randomly assigned into treatment groups, which were either subjected to a novel environment or placed in their respective home-cages to function as baseline controls. Each treatment group was placed in separate cages to prevent the response of one group to influence the response of another. The experiments were repeated several times to rule out cage-specific effects.

The mice were injected (10 ml/kg, i.p.) with vehicle or experimental drug and returned to their home-cage. After 30 min the novelty groups were exposed to a novel environment by placing them in the middle of an open field ($40\times40\times80$ cm^3), situated in a dimly lit room (16.6 lux at the bottom of the field), while the controls stayed in their home-cages. After 5 min the novelty-treated mice were returned to their respective home-cage for another 30 min before being euthanized.

For DOI treatment, the mice were injected with DOI (2 mg/kg, 10 ml/kg, i.p.) or vehicle (10 ml/kg, i.p.) and returned to their cages for 60 min before being euthanized.

All mice were killed by cervical dislocation and the brains quickly removed and dissected on ice. Trunk blood from the same animals were collected in K_2EDTA-coated tubes and centrifuged for 10 min at 3000 *g* to obtain plasma. The blood collection and brain dissection was performed in a room separate from the experiments.

Quantitative assessment of mRNA levels

Total RNA was isolated with Trizol Reagent (Sigma-Aldrich). The RNA samples were dissolved in RNase-free water and RNA was quantified with UV-spectrophotometry at 260 nm. Extracted RNA was reverse transcribed into single-stranded cDNA using the procedure of the supplier (cat.#: A3800, ImProm-II™ reverse transcription system, Promega, Madison, WI, USA). In brief, experimental RNA solution was combined with oligo$(dT)_{15}$ primers and heated at 70 °C for 5 min. The reverse transcription reaction mixture contained 20% ImProm-II™ 5× reaction buffer, 6 mM $MgCl_2$, 0.5 mM dNTP mix and 20 units RNase inhibitor. The reverse transcription reaction was performed at 42 °C for 60 min, followed by heating at 70 °C for 15 min.

The real-time qPCR reactions were performed by adding the sample cDNA to a reaction mixture consisting of 1× Brilliant II SYBR green mastermix (Stratagene, La Jolla, CA, USA) and 15 pmol of each primer (DNA technology, Aarhus, Denmark) and adjusting the volume to 20 μl with DNase free water (Invitrogen, Carlsbad, CA, USA). PCR was performed with a 10 min preincubation at 94 °C followed by 40 cycles of 30 s at 94 °C, 45 s at 60 °C and 1.5 min at 72 °C (Roche Light Cycler 480, Roche, Indianapolis, IN, USA). The PCR primers and method were validated by using serially diluted cDNA to establish a standard curve. To quantify the gene expression of each sample, the C_T value for each sample was obtained and fold change was calculated according to the comparative C_T method as earlier described (Schmittgen and Livak, 2008). For each sample, the amount of targeted mRNA was normalized to that of the reference gene GAPDH. Specific primers designed based on GenBank (NCBI, NIH, MD) data were as follows: *Arc* primers, Forward: 5′-GCA GGT GGG TGG CTC TGA AGA ATA-3′, Reverse: 5′-TCC CGC TTA CGC CAG AGG AACT-3′; *c-fos* primers, Forward: 5′-CAA AGT AGA GCA GCT ATC TCC-3′, Reverse: 5′-CTC GTC TTC AAG TTG ATC TGT-3′; *GAPDH* primers, Forward: 5′-CAT CAA GAA GGT GGT GAA GCA-3′, Reverse: 5′-CTG TTG AAG TCA CAG GAG ACA-3′; *Egr-2* primers, Forward: 5′-TGTTAA-CAGGGTCTGCATGTG-3′, Reverse: 5′-AGCGGCAGTGACATT-GAAG-3′; *CREB* primers, Forward: 5′-ACTGGCTTGGCACAAC-CAGA-3′, Reverse: 5′-GGCAGAAGTCTCTTCATGATT-3′.

Locomotor activity

Locomotor activity while exposed to the open field was recorded by a camera located on the ceiling above the apparatus. The distance the animals moved was analyzed with the video-tracking program EthoVision (version 3.1, Noldus, Wageningen, The Netherlands).

Plasma corticosterone levels

Plasma corticosterone was measured with a commercial ELISA kit (cat.# IB79112, Immuno-Biological Laboratories, Minneapolis, MN, USA). In brief, standards, control and samples were added to the microtiter wells in duplicates and incubated in enzyme conjugate for 60 min. After three rinses with diluted wash solution, the wells were incubated in substrate solution for 15 min at room temperature. Stop solution were added and the OD read at 450 nm within 10 min. Sample corticosterone concentrations were calculated from the standard curve using a four parameter logistics curve fit. The corticosterone standards contain corticosterone concentrations between 0 and 240 nmol/L, and samples were diluted to fit this range.

Data analysis and statistics

The data were analyzed using one-way ANOVA with Tukey's post hoc test. The level of statistical significance was set to $P<0.05$. Data are presented as mean±SEM if not otherwise stated. For RT-qPCR, data from individual experiments were normalized to the novelty- and drug-naïve samples, which were set to 100%.

RESULTS

5-HT_{2A} receptor activation is necessary for FC *Arc* expression following exposure to a novel environment

Five minutes of exposure to a novel environment robustly up-regulated the expression of *Arc* mRNA in FC compared to home-cage controls ($P<0.01$) (Fig. 1a).