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Dopamine concentrations and dopamine receptor gene expression in emotion-related brain structures of female adult rats exposed to stress of chronic isolation from weaning

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Abstract. It is known that early-life stress events induce profound consequences on emotional brain regions including amygdala, involved in emotional processing and the ventral tegmental area (VTA), which contains neuron cell bodies of the mesolimbic dopaminergic system. The aim of this study is to test the hypothesis that stress induced by long-term social isolation from weaning in female rats is associated with alterations in amygdalar dopamine receptor gene expression and VTA dopamine concentrations. Rats were weaned on postnatal day 21 and then exposed to stress of chronic isolation for 9 weeks. Control animals were housed socially. Amygdalar dopamine D_1 but not D_2 receptor gene expression was decreased in isolated rats compared to controls. Dopamine concentrations in the VTA were enhanced following chronic isolation. A negative correlation was observed between amygdalar D_1 gene expression and dopamine concentrations in the VTA. In conclusion, a reduction of dopamine D_1 receptor gene expression in the amygdala in response to stress induced by chronic isolation in female rats was accompanied by an increase in dopamine concentration in the VTA. Further studies are needed to understand the physiological significance, if any, of negative association of amygdalar dopamine receptor D_1 gene expression and dopamine concentrations in the VTA.

Key words: Amygdala — Ventral tegmentum — Neurodevelopment — Long-term stress — Dopamine —Rats

Introduction

It is well known that early-life stress events, e.g. lack of care, child abuse or emotional neglect, induce profound consequences on the organization of different brain circuits, such as emotion-related brain structures underlying stress-coping and adaptation (Daskalakis et al. 2013). The general consensus is that a history of negative early-life events is coupled with increased emotional responding in which the main brain structure involved is amygdala and with impairment of cognitive performance. An important role is played by the ventral tegmental area (VTA), which contains cell bodies of neurons of the mesolimbic dopaminergic system (Douma and de Kloet 2020).

Dopamine acts on dopamine receptors to regulate motor and non-motor functions in a specific manner. Dopamine receptors are classified into 2 families, D1-like receptors and D2-like receptors, on the basis of pharmacologic properties and the ability to regulate the second messenger cyclic adenosine monophosphate (cAMP) generation (Jaber et al. 1996). D_1-like excitatory receptors (D_1 and D_5 receptors) are G_s-protein coupled receptors that activate adenylyl cyclase to produce cAMP with subsequent activation of protein kinase A (PKA). The D_2-like

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inhibitory receptors (D_2, D_3, and D_4 receptors) are G_i-protein coupled receptors, which attenuate the activity of adenylyl cyclase and inhibit the production of cAMP and PKA (Beaulieu et al. 2015).

An elevated dopaminergic function was described in the amygdala of male Wistar rats that underwent early maternal deprivation. No simultaneous changes in the gene expression of dopamine D_1 and D_2 receptors were observed (Rentesi et al. 2013). Chronic isolation from weaning in a rat strain prone to enhanced acquisition of ethanol seeking behaviour (Fawn-Hooded rats) led to increased dopamine D_2 receptor density in the central amygdala (Djouma et al. 2006). To our knowledge, no other data is available on the expression of dopamine receptors in the amygdala of rodents subjected to early life stressors. A decrease in dopamine D_2 receptor gene and/or protein expression was observed in the amygdala of adult male rats exposed to repeated restraint and chronic mild stress in combination with low anxiety trait and amphetamine treatment, respectively (Lehner et al. 2018; Kolosowska et al. 2019). Repeated social defeat stress of adult male mice failed to induce alterations of amygdalar D_2 expression but led to an increase in dopamine D_1 receptor expression in susceptible mice (Huang et al. 2016).

The consequences of long-term stress during development in the form of social isolation of male rats starting from weaning were found to be modified by a lesion of the VTA (Lebedev et al. 1995). In another study with a similar model of social isolation, the authors observed a decrease of dopamine levels in the amygdala but no changes in the VTA (Wang et al. 2012). It is known that dopamine concentrations in the VTA increase in response to acute stressors. Less information is available in respect to repeated and chronic stress. Chronic restraint and repeated social defeat resulted in an increase in spontaneous and burst firing of VTA dopamine neurons (Holly and Miczek 2016). Electrophysiological studies showed that prenatal exposure to maternal immune activation caused an increase in the excitability of dopamine neurons in male, but not in female rats (Csatlosova et al. 2019).

Table 1. Oligonucleotide sequences used in quantitative PCR

Gene	Sense	Sequence 5' → 3'
UQCRFS -reference gene	Forward	ACAGTGGGCCTGAATGTTCC
	Reverse	CACGGCGATAGTCAGAGAAGTC
TfR1- reference gene	Forward	ATACGTTCCCCGTTGTTGAGG
	Reverse	GGCGGAAACTGAGTATGGTTGA
Dopamine D_1 receptor	Forward	CGAACTGTATGGTGCCCTTC
	Reverse	GATGGAATCGATGCAGAATG
Dopamine D_2 receptor	Forward	GCAGTCGAGCTTTCAGAGCC
	Reverse	TCTGCGGCTCATCGTCTTAAG

Brain disorders related to stress and dopamine neurotransmission, such as addictive behaviours, anxiety and depressive disorders are female prevalent (Bangasser and Valentino 2014). Moreover, females showed higher dopamine turnover in the VTA compared to males (Becker and Chartoff 2019). The aim of the present study is to test the hypothesis that stress induced by long-term social isolation during development in female rats is associated with alterations in amygdalar dopamine receptor gene expression and VTA dopamine concentrations.

Materials and Methods

Animals and experimental design

Female Sprague-Dawley rat pups born and handled as described previously (Chmelova et al. 2019a, 2019b) were weaned on postnatal day 21. The pups were exposed to stress of chronic social isolation (housing of one rat *per* cage) or control conditions with social rearing (housing of 3 rats *per* cage) for 9 weeks. The details on animals and experimental design were reported previously (Chmelova et al. 2019a, 2019b). All experimental procedures were approved (approval number Ro-420/15-221) by the Animal Health and Animal Welfare Division of the State Veterinary and Food Administration of the Slovak Republic.

Tissue collection

On postnatal day 85, the animals were decapitated and the brains were procured from the skulls. The amygdala and the VTA were removed, stored in liquid nitrogen and later stored at –70°C until analysed.

Neurochemical analyses

Gene expression of the dopamine receptors D_1 and D_2 was measured in the amygdala by quantitative polymerase chain reaction (qPCR). Total RNA was extracted using TRI Reagent® (Invitrogen, USA) according to the manufacturer's protocol. The mRNA was transcribed into cDNA with the use of ProtoScript First Strand cDNA Synthesis Kit (New-England Biolabs, USA). Gene expression was quantified as described previously (Balagova et al. 2019). Primer BLAST NCBI software was used to design primers specific for studied genes (Table 1).

Dopamine concentrations in the VTA were measured by enzyme-linked immunosorbent assay (Dopamine Research ELISA^{TM} kit; BA E-5300, Nordhorn, Germany) in accordance with manufacturer´s instructions. The tissues of VTA were homogenized in HCl (0.01 N) with sodium metabisulfite (4 mM) and EDTA (1 mM). After centrifugation the

supernatants were collected for measurements. The assay involved special Cis-diol affinity gel extraction, acylation and enzymatic conversion.

Statistical analysis

The obtained data was first checked for distributional properties by Shapiro Wilk's test and subsequently winsorized using a 15% two-tailed quantile trimming to treat the identified outlying observations (1.5 × interquartile range rule) when appropriate. Dopamine receptor D_1 and D_2 gene expression and dopamine concentration data was analyzed by *t*-test for independent groups. The Pearson correlation was used to assess the relationship between the parameters measured. Results are expressed as means ± SEM. The level of significance was set at $p < 0.05$. Data analysis was performed using Statistica 7 software (Statsoft Inc, USA).

Results

Statistical analysis by Student *t*-test showed significantly lower concentrations of mRNA coding for dopamine receptor D_1 in the amygdala ($t_{22} = -2.64$, $p < 0.05$) in stressed rats compared to controls (Fig. 1A). The comparison of concentrations of mRNA coding for dopamine receptor D_2 in the amygdala with those in controls by Student *t*-test did not show any statistical difference (Fig. 1B). Statistical analysis by Student *t*-test showed significantly higher concentrations of dopamine in the VTA ($t_{21} = -2.74$, $p < 0.05$) in stressed rats compared to controls (Fig. 2A).

Pearson correlation analysis (Fig. 2B) showed a significant negative correlation between gene expression of amygdalar dopamine receptor D_1 expressed in arbitrary units and dopamine concentrations in the VTA ($r = -0.4968$, $p < 0.05$). When the correlation analysis was performed in each experimental group separately, no significant correlation was revealed in non-stressed controls ($r = -0.2014$, $p = 0.553$), while there was a marginal significance in the group of rats subjected to the stress of social isolation ($r = -0.5592$, $p = 0.059$). No other significant correlations were found.

Discussion

The long-term isolation from weaning led to a decrease in amygdalar D_1 receptor gene expression in female rats. That represents an original finding as no relevant studies appear to be published previously. The observation of an increase in dopamine D_1 receptor expression in the amygdala of adult male mice induced by chronic defeat stress (Huang et al. 2016), a condition very different from the present study design, is not comparable with the present data. The functional consequences of decreased gene expression of D_1 receptors are unknown. The blockade of dopamine D_1 receptors by an infusion of the D_1 receptor antagonist SCH 23390 into the amygdala reduced the fear expression in rats (Lamont and Kokkinidis 1998). In a more recent report, dopaminergic blockade by the same D_1 receptor antagonist in the central regions of both amygdalas prevented learning related to flavour-taste preference in adult male rats. The effects of the antagonist were abolished by orexin-A administration into the VTA (Risco and Mediavilla 2018). Further studies are needed to be able to relate these findings to neurodevelopmental processes.

The gene expression of dopamine D_2 receptors in the amygdala failed to be influenced by stress induced by social isolation. Several recent studies have shown a relationship between amygdalar D_2 receptors and behavioural and cognitive functions. In dopamine D_2 receptor knockout mice, restora-

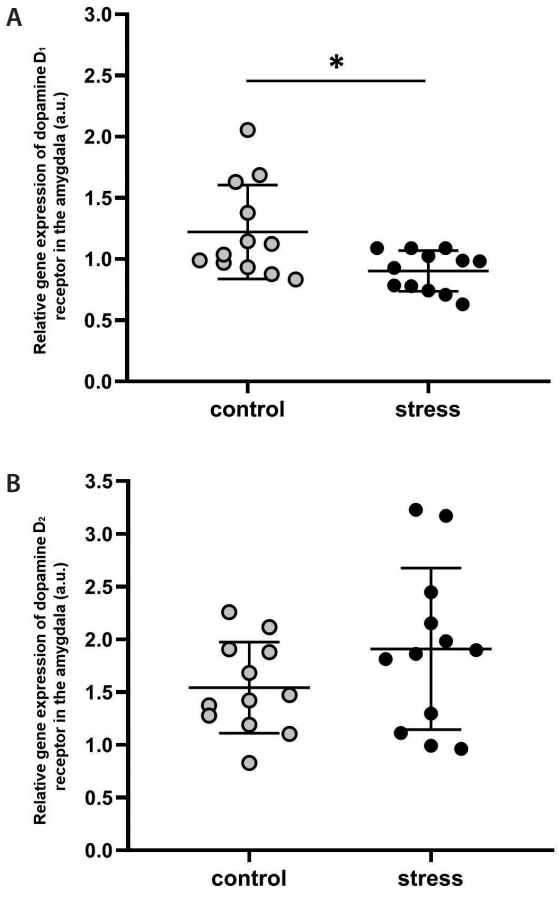

Figure 1. Effect of stress induced by chronic isolation from weaning on amygdalar gene expression of dopamine D_1 receptor (**A**) and dopamine D_2 receptor (**B**). Results are expressed as dot plots with each dot representing an individual subject ($n = 12$ rats *per* group) with the mean ± SEM represented by horizontal lines. Statistical analysis was performed by *t*-test for independent groups: * $p < 0.05$.

tion of D_2 receptor gene expression in the central amygdala normalised their enhanced impulsivity (Kim et al. 2018). Experiments with dopamine D_2 receptor antagonist and/or agonist infused into the basolateral amygdala revealed that these receptors play a role in fear extinction (Shi et al. 2017) and stress-induced memory impairment (Keshavarzian et al. 2018). The mentioned experiments were performed in male rats. Future studies in female animals are needed to look for potential changes in D_2 receptor gene expression in the subregions of the amygdala in response to long-term isolation.

Long-term isolation of rats from weaning resulted in a significant increase in dopamine concentrations in the VTA. This is consistent with the well-established rise in dopamine release from the VTA in animals exposed to acute stress stimuli (Holly and Miczek 2016). However, present studies did not use acute stress stimuli. The study by Wang and colleagues (2012) who were working with a similar model of chronic isolation lasting a comparable duration, did not observe any changes in dopamine concentrations in the VTA but they Wistar male rats. We suggest that the enhanced dopamine concentrations found in female rats in this study may be due to an influence of sex and rat strain.

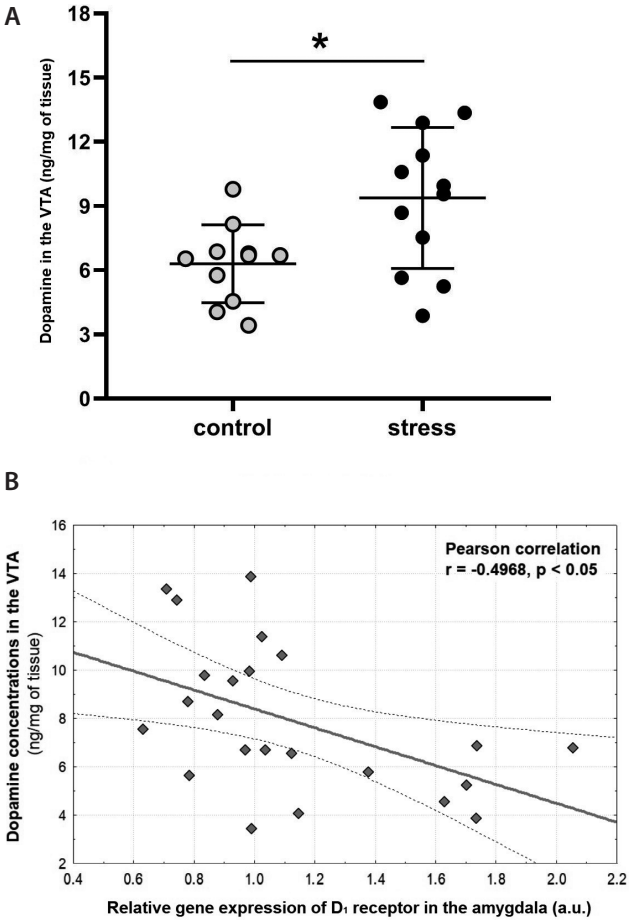

Figure 2. A. Effect of stress induced by chronic isolation from weaning on dopamine concentrations in the VTA. Results are expressed as dot plots with each dot representing an individual subject (n = 12 rats *per* group) with the mean ± SEM represented by horizontal lines. Statistical analysis was performed by t-test for independent groups: * $p < 0.05$. **B.** Negative correlation between the gene expression of dopamine D_1 receptor in the amygdala and the dopamine concentrations in the VTA. Statistical significance was evaluated by Pearson analysis.

We have observed a negative correlation between amygdalar dopamine receptor D_1 gene expression and dopamine concentrations in the VTA, which was particularly evident in rats exposed to stress of chronic isolation. Obviously, this does not imply their causal relationship. We may, however, speculate that chronic isolation affected the pathway from the basolateral amygdala to the dentate gyrus. This pathway was found to be modified by VTA lesion (Abe et al. 2008). Moreover, attenuation of long-term potentiation induced by VTA lesion was restored by dopamine D_1 and D_2 receptor agonists injected into the basolateral amygdala (Abe et al. 2009). Functional disconnection of central amygdala from the VTA facilitated associative learning procedures (El-Amamy and Holland 2007). There are few other possible physiological mechanisms, which can be considered based on the published data. D_1 receptors in the amygdala were shown to modulate neuronal excitability in this brain region (Floresco and Tse 2007). The projections from amygdala into the VTA may primarily reach gama-aminobutyric acid (GABA) rather than dopamine neurons (Beier et al. 2015). It is therefore possible that D_1 receptors in the amygdala activate VTA-projecting neurons, which in turn stimulate GABA cells of the VTA, and that finally results in inhibition of dopamine neurons. Consistently, an increase in amygdalar D_1 expression may lead to reduced dopamine release in the VTA.

In conclusion, the present study revealed that dopamine D_1 receptor gene expression in the amygdala was reduced in response to the stress induced by chronic isolation in female rats. This change was accompanied by an increase in dopamine concentration in the VTA. Further studies are needed to understand the physiological significance, if any, of negative association of amygdalar dopamine receptor D_1 gene expression and dopamine concentrations in the VTA.

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