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Chronic postnatal monoamine oxidase inhibition affects affiliative behavior in rat pups

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ABSTRACT

Monoamine neurotransmitters serotonin (5-HT), dopamine (DA), and noradrenaline (NA) act as important modulators of mammalian brain development and represent neurobiological substrates of affiliative behavior reflected in rat pups as a tendency to huddle or produce ultrasonic vocalizations (USV) when separated from the nest. Monoamines are metabolized through oxidative deamination catalyzed by the mitochondrial enzyme monoamine oxidase (MAO). In this study, we examined the consequences of postnatal MAO inhibition on affiliative behavior in rat pups. Pups received daily injections of either an irreversible non-selective MAO inhibitor tranylcypromine (TCP) or saline, from post-natal day (PND) 1 to PND 22. Quantitative and qualitative components of USV were analyzed on PNDs 10, 13 and 16 in order to determine the level of separation-induced anxiety and the modality of vocal communication. In comparison to control pups, TCP-treated pups displayed higher cortical 5-HT, DA and NA levels, higher peripheral 5-HT concentration, lower body mass throughout the pre-weaning period, higher isolation-induced drop in body temperature, and reduced total number of calls. Furthermore, they produced lower pitched calls of longer average duration without a preferable waveform. Our results demonstrate that chronic MAO inhibition by TCP primarily affects 5-HT concentrations, but also raises central catecholamine levels. They further indicate that disturbed monoaminergic homeostasis during early postnatal development leads to decreased weight-gain, compromised thermoregulation, and altered affiliative behavior in pre-weaning pups as reflected in reduced separation anxiety and inadequate vocal communication. Finally, they suggest a need for thorough examination of the potential effects of TCP and other monoamine inhibitors on the developing human brain.

1. Introduction

Monoamine neurotransmitters act as important neuromodulators of mammalian brain development, and alterations in serotonin (5-HT), dopamine (DA), and noradrenaline (NA) neurotransmission during prenatal and early postnatal periods are considered to affect the development of essential cortical circuits required for functional neuronal signaling and the resulting behavioral outcomes (Benes et al., 2000; Thompson and Stanwood, 2009).

Monoamines, together with neuropeptides and endogenous opioids, represent neurobiological substrates of affiliative behavior. NA is involved in early postnatal offspring and maternal social learning (Nelson and Panksepp, 1998), the DA reward system promotes maternal behavior (Stoesz et al., 2013), while 5-HT regulates neural networks necessary for motivation, memory, sensory processing, and other integral processes of social interaction (Insel and Winslow, 1998). In mammalian infants, affiliation is reflected in tendency to grasp, huddle, or produce a high-pitched, species-specific alarm cry when separated from their family group.

Affiliation of a pre-weaning rat with its dam and littermates is dominantly mediated by thermal and tactile sensory inputs, and removal of a pup from its social group elicits behavioral activation, including ultrasonic vocalization (USV), which induces maternal search and retrieval behavior (Insel and Winslow, 1998; Nelson and Panksepp, 1998). Growing evidence has implicated the role of monoamines in the modulation of USV. Dopamine receptor agonists (Dastur et al., 1999) and reuptake inhibitors (Kehoe and Boylan, 1992) are shown to significantly reduce USV. Noradrenaline has been reported to have the opposite effect as both, the α_2 receptor agonists (Hård et al., 1988) and NA reuptake inhibitors (Winslow and Insel, 1990a) increase USV. 5-HT influence on USV seems to be more complex: 5-HT enhancers acting on the 5-HT transporter (Hodgson et al., 2008; Olivier et al., 1998; Winslow and Insel, 1990b) as well as 5-HT_{1A} and 5-HT_2 receptor

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); DA, dopamine; NA, noradrenaline; USV, ultrasonic vocalization; MAO, monoamine oxidase; TCP, tranylcypromine; PND, post-natal day; ADR, adrenaline; LFC, latency to first call; TNC, total number of calls; ACD, average call duration; FME, frequency of maximal energy.

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agonists reduce USV, 5-HT_{1B} agonists enhance USV, while 5-HT_3 receptor agonists display no effect (Olivier et al., 1998).

Monoamines are metabolized through oxidative deamination catalyzed by the mitochondrial enzyme monoamine oxidase (MAO) which comes in two isoforms expressed in brain and peripheral tissues. 5-HT and NA are preferentially oxidized by MAO A, while DA represents an equally-preferred substrate for both, MAO A and MAO B (Billett, 2004). Efficient inhibition of both MAO isoforms can be achieved by application of irreversible non-selective MAO inhibitors, which are clinically used in treatment of atypical, bipolar, or treatment-resistant depression (Fiedorowicz and Swartz, 2004).

In this study, we examined the consequences of early postnatal MAO inhibition on affiliative behavior in rat pups. Pups received daily injections of either an irreversible non-selective MAO inhibitor tranylcypromine (TCP, 2 mg/kg s.c.) or saline, from post-natal day (PND) 1 to PND 22. Body mass and temperature were measured in order to check the influence of treatment on weight gain and thermoregulation. Quantitative and qualitative components of ultrasonic vocalization were analyzed on PNDs 10, 13 and 16 in order to determine the level of separation-induced anxiety and the modality of vocal communication. Finally, concentrations of 5-HT, NA and DA in the frontal cortex and blood serum, as well as serum adrenaline (ADR) concentration, were measured at the end of treatment in order to search for the biochemical basis of the observed behavioral changes.

2. Materials and methods

2.1. Animals

Wistar females were mated with males of the same strain in the animal facility of the Croatian Institute for Brain Research (University of Zagreb, Croatia). Three females arrived from the Facility during the second week of pregnancy. Two days before parturition, females were separated and remained singly housed until the end of the experiment at postnatal day (PND) 22. The animals were housed in polycarbonate cages under 12 h light: 12 h dark conditions at 22 ± 2 °C, with free access to rat chow and tap water.

The study was approved by the Ethics committee of the University of Zagreb and was conducted in accordance with the Directive of the European Parliament and of the Council (2010/63/EU) and the Croatian Animal Protection Law ("Narodne Novine", 135/2006 and 37/2013). All efforts were made to reduce the number of animals used and to minimize animal suffering.

2.2. Pharmacological treatment

The day on which pups were born was considered as PND 0. In each litter, pups were weighed, sex-determined and assigned to either experimental or control group (care was taken to have similar gender ratio and average body mass in both groups) on PND 1. Pups were marked with marker pen of two different colors, on one of the hind limbs until PND 10, and on the tail afterwards. Marks were renewed daily, immediately after injections. A total of 17 pups (7 males, 10 females) were assigned to the control and 18 pups (7 males, 11 females) to the experimental group. The experimental (TCP) group of pups received subcutaneous injections of 2 mg/kg tranylcypromine (Sigma-Aldrich, St. Louis, MO, USA) in the nape, from PND 1 until PND 22. TCP was dissolved in ethanol and saline, neutralized with HCl to a pH around 7 and, before treatment, warmed to body temperature. Solutions were delivered in volumes of 3.3 mL per kg of body mass, by a 50 μL glass syringe (Hamilton) with disposable 30G needles (BD, Drogheda, Ireland), until pups reached 15 g, and in volumes of 5 mL per kg of body mass, by disposable 0,5 mL plastic syringes with 30G needles (BD Micro-Fine Plus), until the end of treatment. The control group was treated with saline in the same manner. All injections were performed between 10 and 11 a.m.

2.3. Body mass and temperature measurements

Pups were weighed every day before injections. Body temperature was measured on days of USV testing (PNDs 10, 13 and 16), immediately after weighing and immediately after USV testing. In order to minimize stress and discomfort of pups, axillary temperature was measured with a contact thermometer with 0.1 °C intervals, containing a thin tip which enabled body temperature reads within 10–20 s.

2.4. USV testing

Separation-induced ultrasonic vocalization was measured on PNDs 10, 13 and 16, between 4 and 5 p.m. Immediately after separation from the litter, animals were individually placed in a Styrofoam box (27,4 × 26,7 × 18,6 cm in dimension covered with black adhesive paper on the inside) with the ultrasonic microphone (Dodotronic ultramic 250K) placed 10 cm above the animal. Vocalizations were recorded for 75 s using Seawave software (CIBRA) with a sampling rate of 250 kHz, format 16 bit. Room temperature was kept at 21 °C, and the apparatus was cleaned after each pup.

Recordings were acoustically analyzed using BatSound Pro Software (version 3.0). Parameters analyzed for each test included: latency to first call (LFC), total number of calls (TNC), average call duration (ACD), and frequency of maximal energy (FME). Analysis of waveform patterns of calls was performed in the sonograms collected on PND 13. According to the shape, waves were divided into the following 8 categories (Table 1): 1) short single-frequency calls ("dots", of <20 ms duration); 2) single frequency calls; 3) simple frequency sweeps (predominantly rising); 4) double frequency sweeps (predominantly in fall-rise order); 5) multiple frequency sweeps; 6) combination of the two waveforms appearing at the same time at two different frequencies (predominantly 1 with 2–5); 7) multiple waveform combination (combination of 3 or more waveforms appearing at the same time at different frequencies); and 8) complex broadband wave.

2.5. Sample collection

Pups were sacrificed at PND 22. Four hours after the last injection, a pup was separated from the litter, carried in a small cage to the laboratory and put into an exicator containing a cotton pad soaked with isoflurane (Abbott). Anesthesia was maintained by applying a small beaker with a cotton swap soaked in isofluorane to the snout. About 1.5 mL of blood was withdrawn from the *vena cava* into an anticoagulant-free syringe and immediately transferred into a microtube. Animal was then decapitated and the brain was removed from the scull and placed on a cold plate. A 5 mm coronal cut was made at the frontal lobes, and cortex encompassing all cortical areas anterior to bregma was peeled off. Samples were weighted and frozen at −80 °C for later analysis.

2.6. Measurement of monoamine concentrations

Due to the limited number of wells in ELISA plates, serum and cortical monoamine concentrations were measured in blood and brain samples of 12 TCP-treated (6 males, 6 females) and 12 saline-treated (6 males, 6 females) pups. For each subgroup, blood samples were selected on the basis of serum quality and quantity, and brain samples of the same animals were then taken for cortical measurements. Blood samples were allowed to clot and serum was separated by centrifugation 10 min at 500 × *g*. The frozen cortical samples were thawed and homogenized in 4 volumes (w/v) of a solution of 0.01 N hydrochloric acid containing 1 mM EDTA and 4 mM Na_2S2O_5. Tissue homogenates were then centrifuged at 24,000 × *g* for 20 min at 4 °C, and aliquots of the clear supernatant were used for the measurements.

5-HT concentration in cortex and serum was determined using the Serotonin Research ELISA kit; DA, NA and ADR concentrations in