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EFFECTS OF AN EARLY EXPERIENCE OF REWARD THROUGH MATERNAL CONTACT OR ITS DENIAL ON THE DOPAMINERGIC SYSTEM OF THE RAT BRAIN

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Abstract—The mesolimbic/mesocortical dopaminergic pathway plays a pivotal role in the reward system. During the neonatal period the mother is the main source of rewarding stimuli. We have developed an experimental model in which rat pups learn a T-maze during the neonatal period (postnatal day (PND) 10–13) using contact with the mother as the reward. One group of animals is allowed contact with the mother (receipt of expected reward, RER) while the other was denied (denial of expected reward, DER). We determined the effects of these two early experiences in the prefrontal cortex (PFC) and the nucleus accumbens (nAc), the levels of dopamine (DA) and its metabolites [3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] by high-performance liquid chromatography and those of D1 and D2 receptors by autoradiographic *in vitro* binding both on PND 13 and in adulthood. On PND13, 2 h after the end of training, the RER experience resulted in higher DA, HVA and D1 receptor levels in the nAc, while the DER in lower DA and its metabolites (DOPAC and HVA) in the PFC. These results could be related to the reward the RER pups received through the contact with their mother. The RER and DER early experience had long-term sex-dependent effects: The RER-induced activation of the dopaminergic system in the nAc was also evident in adult female rats. In contrast, adult DER males, similar to PND13 animals, had reduced dopamine in the PFC. Our results document that early experiences, a key determinant of adult brain function, affect the dopaminergic system which is disturbed in many psychiatric diseases.

Key words: early-life experiences, dopamine and its metabolites, D1 receptors, prefrontal cortex, nucleus accumbens, reward.

INTRODUCTION

The dopaminergic system is a key neuromodulator implicated in a variety of neuropsychiatric disorders such as schizophrenia, major depression, drug addiction, and attention-deficit hyperactivity disorder (ADHD) (Melis et al., 2005; Krishnan and Nestler, 2008; Howes and Kapur, 2009; Genro et al., 2010). Under physiological conditions, it plays a cardinal role in a variety of important brain functions such as reward, cognition, motor control, emotion, sexual and maternal behavior, aggression, and pair bonding (Nieoullon and Coquerel, 2003; Wise, 2004; Salamone et al., 2005; Berridge, 2007; Yanowitch and Coccaro, 2011; Burkett and Young, 2012). The process of reward, which is mediated by the mesolimbic/mesocortical dopamine system, is a basic component underlying many of the above behaviors by providing the necessary drive for learning on one hand and the consummatory element of emotionally laden behaviors such as sexual and maternal behavior. The mesolimbic/mesocortical dopamine system consists of dopaminergic neurons localized in the ventral tegmental area which project to brain areas such as the nucleus accumbens (nAc) and the prefrontal cortex (PFC). Furthermore, there is a feed-in circuit in the system, since the PFC can modulate the release of dopamine (DA) in the nAc (Jackson et al., 2001; Del Arco and Mora, 2008), the brain area which mediates the effects of natural and artificial rewards (Wyvell and Berridge, 2000; Hajnal et al., 2004; Berridge, 2007). Moreover, the overall circuit plays a pivotal role in the "wanting" and seeking part of the reward processes (Berridge, 2007).

It is well known that early-life experiences have profound long-term effects on adult brain function and behavior (Heim et al., 2004; Pryce et al., 2005; Champagne and Curley, 2009) and are often associated with an increased risk for the development of psychiatric disorders (Heim and Nemeroff, 2001; Schmid et al., 2011). A large body of data obtained in humans as well as in animal studies have shown that early-life events which interfere with or disrupt mother–infant interactions, alter the development and the activity of

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Abbreviations: ADHD, attention-deficit hyperactivity disorder; ANOVA, analysis of variance; CTR, control; D1, type 1 dopamine receptors; D2, type 2 dopamine receptors; DA, dopamine; DER, denial of expected reward; DOPAC, 3,4-dihydroxyphenylacetic acid; HPLC, high-performance liquid chromatography; HVA, homavanillic acid; IL, infralimbic cortex; nAc, nucleus accumbens; PFC, prefrontal cortex; PND, postnatal day; PrL, prelimbic cortex; RER, receipt of expected reward; RT, room temperature; SEM, standard error of the mean; VTA, ventral tegmental area.

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the mesolimbic and mesocortical dopaminergic systems (Cabib et al., 1993; Galvin et al., 1995; De Bellis et al., 1999; Hall et al., 1999; Matthews et al., 2001; Kosten et al., 2003, 2005; Brake et al., 2004; Björklund and Dunnett, 2007; Fone and Porkess, 2008; Strathearn, 2011; Lovic et al., 2013). For example it has been shown that neonatal handling reduces accumbal DA release after stress and leads to sex dimorphic alterations of DA levels and its turnover in different brain regions (Papaioannou et al., 2002) while it increases D1 receptor density in the nAc (Gariépy et al., 2002). It has also been shown that prolonged maternal separation and isolation of rat pups leads to modified levels of D1 and D2 receptors in the striatum and the PFC, as well as elevated basal and stress-induced dopamine levels while decreased DAT levels in the striatum in adulthood (Hall et al., 1999; Meaney et al., 2002; Lejeune et al., 2013; Rentesi et al., 2013). Furthermore, repeated maternal separation results in elevated striatal concentrations of DA and a reduced turnover of this neurotransmitter in the PFC (Matthews et al., 2001). Moreover, adult offspring of high-licking and grooming mothers exhibit alterations of dopamine levels in the medial PFC in response to stress (Zhang et al., 2005). Thus, the dopaminergic system seems to be quite sensitive to early-life manipulations and environmental factors (Meaney et al., 2002; Björklund and Dunnett, 2007; Oreland et al., 2011; Lovic et al., 2013; Ventura et al., 2013).

In our laboratory we have developed a new experimental paradigm in which rat pups during postnatal days (PNDs) 10–13 are exposed to a T-maze, one arm of which leads to the mother-containing cage. One group of animals is allowed access to the mother (receiving expected reward, RER) whereas the other is not (denied the expected reward, DER) (Panagiotaropoulos et al., 2009). Since the dopaminergic system is an important component of the reward system and it appears to be sensitive to early-life experiences, in the present study we investigated both the short- and long-term effects of our model on the dopaminergic system. Thus, we determined in both the PFC and the nAc of male and female rats, the levels of dopamine (DA) and its metabolites by high-performance liquid chromatography (HPLC) and those of D1 and D2 receptors by autoradiographic *in vitro* binding on PND13, after the neonatal experience of the T-maze training, and in adulthood.

EXPERIMENTAL PROCEDURES

Animals

Wistar rats of both sexes born and reared in our colony were used in these experiments. Animals were kept under standard conditions (24 °C, 12:12 h light/dark cycle) and received food (Kounker-Keramari bros. & Co., Athens, Greece) and water *ad libitum*. Prior to the day of birth, postnatal day 0 (PND0), each litter was randomly allocated to either of the two neonatal experience groups [pups denied (DER) or receiving (RER) the expected reward of maternal contact during training in a T-maze, see below] or to the control (CTR) group. Wood chip was added every 4–5 days into the cages, without disturbing either the litter or the dam. On PND22, animals were weaned and housed in same-sex, same group (DER, RER, CTR) cages with three to four animals per cage. All animals were left undisturbed (with the exception of weekly cage cleaning) until adulthood (3 months old).

Two different cohorts of animals were used: One cohort of animals was used for the neurochemical experiments during the neonatal period [postnatal days 10–13 (PND10–13)]. A second cohort of animals was used for the neurochemical experiments in adulthood. In each of these two cohorts, for each group (DER, RER, CTR) five litters were used; from each litter two male and two female animals were used for HPLC while one male and one female for D1 and D2 autoradiographic *in vitro* binding. Estrous cycle was not determined in the female animals used in the study, to avoid the stress of vaginal smear collection. However, vaginal smears were taken from their littermates (animals not included in the experiments). All females sampled had normal 4- to 5-day non-synchronized, estrous cycles. Moreover, the estrous cycle phases were equivalently distributed in animals from the three groups (DER, RER, and CTR). Thus any differences detected between groups could not be ascribed to differences in the phase of the estrous cycle. All experiments were carried out in agreement with ethical recommendations of the European Communities Council Directive of 22 September, 2010 (2010/63/EU). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Neonatal training in the T-maze

Briefly, all animals of each litter were exposed either to the RER or DER experience, starting from PND10 until PND13. As previously described in detail (Panagiotaropoulos et al., 2009; Diamantopoulou et al., 2011), we used a custom-made T-maze. At the end of one of its two arms a small sliding door (9 × 11 cm) permitted access to the mother-containing cage when pups were trained under continuous reinforcement (RER) or remained always closed, preventing entrance into the cage, when pups were trained under the condition of denial of expected reward (DER). At the end of the other arm of the T-maze another cage was placed, without access from inside the T-maze, containing a virgin female rat for control purposes. Each pup from each group (DER, RER) was subjected to 10 trials of a maximum of 60 s duration per day. If a pup did not reach the entrance of the mother-containing cage within the 60-s max duration of each trial, the experimenter gently guided it to the entrance. In the case of RER training once the pup either reached the entrance of the mother-containing cage or was guided there, it would enter the cage and then the next pup was exposed to the same procedure. When all pups of a litter performed the first trial of the RER training, the same procedure was repeated for the next trial until all pups had performed 10 trials. On the other hand, during