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# Association between body weight of newborn rats and density of serotonin transporters in the frontal cortex at adulthood

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**Summary.** Persisting alterations in monoaminergic innervation patterns have been observed following various environmental manipulations and neuro-psychopharmacological treatments during fetal or early postnatal life. The present study investigates the question how differences in initial growth conditions at birth might interfere with subsequent development of both serotonergic and noradrenergic innervation in the rat frontal cortex (FC) and brain stem. For this purpose, newborn rat littermates were divided into two groups, a low and a high birth weight group, and the densities of both serotonin (5-HT) and norepinephrine (NE) transporters in the FC and brain stem were analyzed at adulthood. 5-HT transporter density in the FC was significantly higher in the high birth weight group as compared with the low birth weight group. No significant differences were observed between both groups in the density of 5-HT transporters in the brain stem and in the densities of NE transporters in FC and brain stem. It is discussed that differences in birth weight may affect the postnatal development of 5-HT projections to the frontal cortex.

**Keywords:** Birth weight, brain development, serotonin transporter, frontal cortex, brainstem.

## Introduction

The influence of high or low birth weight on subsequent brain development is still poorly understood. Nevertheless, there is some evidence that low birth weight resulting from placental insufficiency, fetal malnutrition and fetal growth retardation is associated with an elevated probability of developing a variety of mental disorders, e.g. cognitive impairment (Liu et al., 2000; Olness, 2003), schizophrenia (Gunnell et al., 2003; Hultmann et al., 1999; Matsumoto et al., 2001), depression (Thompson et al., 2001), and attention deficit hyperactivity disorder (Bhutta et al., 2002; Mick et al., 2002; Saigal et al., 2003). Especially alterations of monoaminergic systems are assumed to play an important role in these psychiatric disorders (Dean, 2003; Hawi et al., 2002; Kent et al., 2002; Powers, 1999; Sawa and Snyder, 2002). The development of these systems has been shown to be affected by intrauterine growth conditions. Experimentally induced transient intrauterine growth retardation leading to reduced birth

weight of both the whole body and the forebrain caused increased levels of norepinephrine (NE), dopamine, serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) immediately after birth, with concentrations of serotonin and 5-HIAA remaining elevated in the forebrain and brain stem until at least postnatal day 15 (Minkowski et al., 1981).

The present study was performed to evaluate probable associations between naturally occurring differences in birth weight and the development of monoaminergic projections to the frontal cortex (FC). For this purpose, newborn male rat pups with birth weights being either below or above average of all male littermates were raised, and the densities of 5-HT and NE transporters were measured in two brain regions, the brain stem and the frontal cortex at adulthood.

## Materials and methods

### *Animals*

Animal experiments were performed in accordance with German laws for the care and use of laboratory animals (as approved by the Bezirksregierung Braunschweig, License No. 604.42502/01-24.92).

Wistar Kyoto rats were obtained from a commercial breeder (Harlan-Winkelmann GmbH, Borchen, Germany) and used for further breeding in our own environmentally conditioned animal facility under standardized conditions of temperature and lighting. After mating, the dams were housed in single standard laboratory cages with free access to food and water. Only litters consisting of at least 8 newborns with at least 4 males were included in this study. Within 24 hours after birth, male pups were weighed (further called birth weight). The two male pups with the highest and the two with the lowest birth weight were chosen out and all the other pups were removed. Within the remaining four pups one of the pups with the lower birth weight and one with the higher birth weight were related to each other and called a 'pair' if the difference between birth weight was at least 10% but not more than 20%. If, in one litter, there was just one pair of male pups fulfilling these criteria, the other two pups were chosen to bring the resulting number of pups to four. But then these two pups were removed from further analysis. If, in one litter, there was no pair fulfilling the criteria in birth weight, the whole litter was removed. Thus, each dam reared four male pups of her own litter. Pups were marked by tattooing small dots into the ears.

Until weaning dams were housed with their four pups together. At day 25 the dams were removed and the pups left together until puberty. Body weights were monitored weekly, but any additional manipulations were avoided. At day 90, pairs not fulfilling the criteria of birth weight were removed. The others were decapitated after deep $CO_2$-narcosis between 11.00 and 12.00 a.m., and the brains were quickly removed and frozen on dry ice. After decapitation, we recognized that one litter contained a hydrocephalic rat and thus, the whole litter has been removed from further analysis.

By this procedure, 6 dams reared 24 male pups of which 7 pairs fulfilled the criteria of differences in birth weight. 5 dams reared one pair fulfilling the criteria and one pair used to fill up litter size, whereas just one dam reared two pairs fulfilling the criteria. Thus, finally 12 brains of 6 pairs of animals fulfilling the criteria of differences in birth weight were used for further analysis.

### *Sample preparation*

Crude membrane preparations of frontal cortex and brain stem were prepared as described previously (Moll et al., 2000, 2001; Wegerer et al., 1999). Brain regions were dissected from frozen brains, homogenized by sonification in ice-cold PBS (10 mM potassium phosphate [pH 7.4], 154 mM NaCl) containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.02% thimerosal, and spun down 30 min at 40,000 × g, 4°C. Pellets were re-suspended in ice-cold sample buffer (50 mM Tris-HCl [pH 7.7], 120 mM NaCl). After low speed centrifugation (15 min at 1,000 × g, 4°C), the supernants were centrifuged again for 30 min at 40,000 × g, 4°C, and the resulting pellets were washed twice with sample buffer, and finally re-suspended in sample buffer at a concentration of 60 mg wet weight/ml.

### *[$^3$H]-paroxetine and [$^3$H]-nisoxetine binding assays*

All transporter ligand binding assays were performed as described previously (Moll et al., 2000, 2001; Wegerer et al., 1999).

For measurements of [$^3$H]-paroxetine binding, aliquots of the membrane preparations were suspended in buffer A (50 mM Tris-HCl [pH 7.7], 120 mM NaCl, 5 mM KCl) at a concentration of 20 mg wet weight/ml. Of each of this membrane suspensions, two 100 µl aliquots were mixed with 100 µl buffer A containing 0.3 nM or 3 nM [$^3$H]-paroxetine (specific activity: 20.2 Ci/mmol), respectively, and with 100 µl buffer A, to give a final concentration of [$^3$H]-paroxetine of

0.1 or 1 nM, respectively. To measure unspecific binding, 5-HT was added to two parallel samples at a final concentration of 75 µM. Samples were incubated for 60 min at 25°C under vigorous shaking.

For measurements of [$^3$H]-nisoxetine binding, aliquots of the membrane preparations were suspended in buffer B (50 mM Tris-HCl [pH 7.7], 300 mM NaCl, 5 mM KCl) at a concentration of 20 mg wet weight/ml. Of each of this membrane suspensions, two 100 µl aliquots were mixed with 100 µl buffer B containing 1.2 nM or 12 nM [3H]-nisoxetine (specific activity: 20.2 Ci/mmol), respectively, and with 100 µl buffer B, to give a final concentration of [$^3$H]-nisoxetine of 0.4 or 4 nM, respectively. To measure unspecific binding desipramine was added to two parallel samples at a final concentration of 33.3 µM. Samples were incubated for 60 min at 25°C under vigorous shaking.

After incubation, the reaction mixtures were filtered through prewet Wathman GF/B filters, presoaked in 0.05% polyethyleneimine, using a 12-channel cell harvester, and filters were washed with 20 vol. buffer A or 60 vol. buffer B, respectively. The radioactivity trapped by the filters was determined by liquid scintillation spectroscopy. All binding assays were performed at least in triplicates.

Protein concentrations in membrane suspension of 20 mg wet weight/ml were measured by the method of Lowry et al. (1951).

### *Data analysis and statistics*

The affinity and capacity parameters ($K_D$- and $B_{max}$-values) of [$^3$H]-paroxetine and [$^3$H]-nisoxetine binding were derived from estimated *Scatchard* plots of saturation isotherms of specific binding data by linear connection of the two measured points. This was possible because our previous data (Moll et al., 2000, 2001; Wegerer et al., 1999) have shown a single straight unbroken line in *Scatchard* derived from data of ligand concentrations between 0.05 to 4.00 µM or between 0.01 to 1.00 µM for [$^3$H]-nisoxetin or [$^3$H]-paroxetin, respectively. Data were expressed as means ± SD. Differences between the two groups were statistically analyzed by two-tailed *Wilcoxon*-tests for paired samples.

## Results

### *Birth weight and growth parameters*

Mean birth weight was 9.14 ± 0.97 g and 10.45 ± 1.16 g in the lower and higher birth weight group, respectively (Fig. 1). The difference between both groups reached a level of significance of $p = 0.028$, according to two-tailed *Wilcoxon*-test for paired samples. The percentage differences between pubs with lower and higher birth weight in each litter ranged between 10.1% and 18.6% with a mean size difference of 14.3 ± 3.0%.

The influence of low or high birth weight on the later gain of body weight until adulthood is shown in Fig. 2. Both curves represent

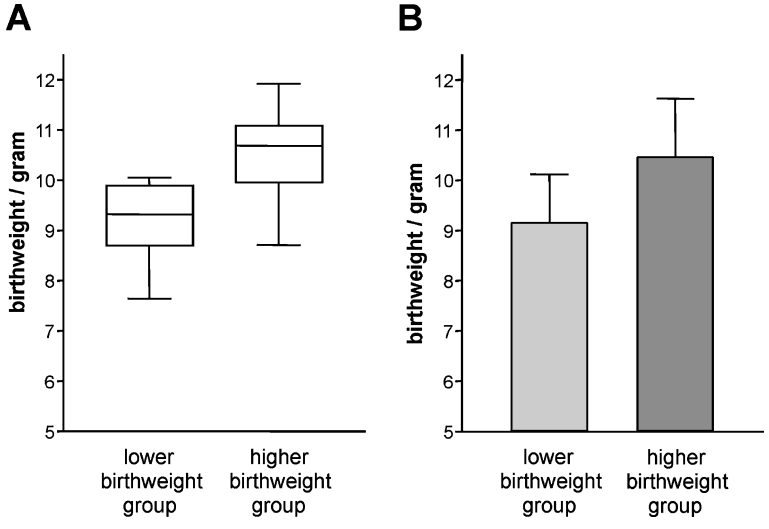


**Fig. 1.** Newborn male rat pups were designated to two weight groups, a low birth weight group and a high birth weight group, if their initial body weight was at least 10%, but not more than 20% different from each other. Differences in birth weight between both groups are shown as box-plot (**A**) and mean ± SD (**B**), n = 6 per group

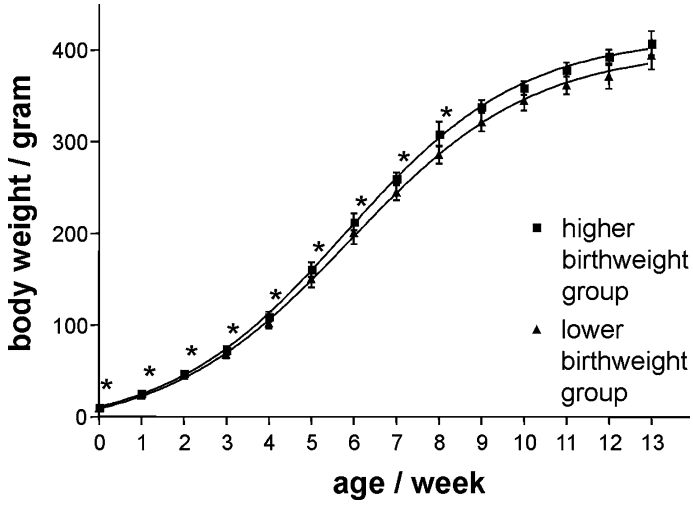


**Fig. 2.** Body weights of the lower and the higher birth weight group are shown as mean ± SD over the entire experimental period (13 weeks). Differences were statistically analyzed by two-tailed *Wilcoxon*-tests for paired samples. $^{*}p < 0.05$. n = 6 per group

typical sigmoid growth patterns. Until the age of eight weeks, differences remained significant ($p < 0.05$). At the endpoint of this study, at the age of 13 weeks, the bodyweight in the lower birth weight group was still lower than that of the higher birth weight group ($393.5 \pm 36.0$ g vs. $407.3 \pm 31.9$ g), but this difference was no longer significant.

### *Transporter densities*

The estimated affinity parameters ($K_D$-values) of the binding sites for both ligands in the frontal cortex and the brainstem were rather similar. No differences were noticed in the $K_D$-values measured in these two regions between the two groups of birth weight.

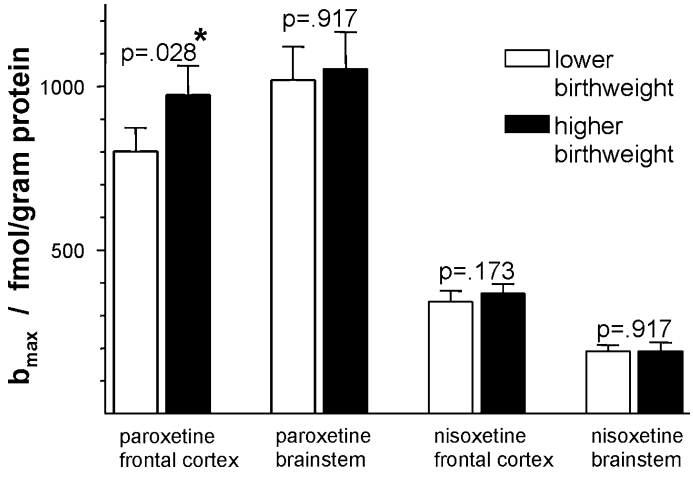


**Fig. 3.** Densities of 5-HT and NE transporters in frontal cortex and brain stem, shown as [$^3$H]-paroxetine and [$^3$H]-nisoxetine binding, respectively. $B_{max}$-values are shown as means ± SD for both the lower and higher birth weight groups. Differences were statistically analyzed by two-tailed *Wilcoxon*-tests for paired samples. n = 6 per group

The measured densities of 5-HT and NE-transporters ($B_{max}$-values of [$^3$H]-paroxetine and [$^3$H]-nisoxetine binding, respectively) in the frontal cortex and the brainstem of rats with lower or higher birth weights are summarized in Fig. 3. ANOVA revealed an effect of birth weight group on $B_{max}$-values of [$^3$H]-paroxetine binding in the frontal cortex ($F(1,5) = 18.64$) with rats of the lower birth weight group presenting a significantly lower 5-HT transporter density than rats of the higher birth weight group ($780 \pm 127$ fmol/mg vs. $974 \pm 216$ fmol/mg; two-tailed *Wilcoxon*-test for paired samples, $p = 0.028$). No significant effects of birth weight group on the densities of other ligand binding sites were observed, neither on the [$^3$H]-nisoxetine binding in the frontal cortex nor on the [$^3$H]-paroxetine or [$^3$H]-nisoxetine binding in the brainstem.

## Discussion

The results of the present study show that initial growth differences of rat littermates at birth are associated with different densities of serotonergic transporters in the frontal cortex (FC) at adulthood. Monoamine transporters specific for each monoaminergic system are located at dopaminergic, noradrenergic and serotonergic presynapses (Hoffman et al., 1998) and serve to terminate the action of released neurotransmitter in the synaptic cleft. Therefore, these transporter proteins are appropriate markers for the integrity and density of monoaminergic innervation. Thus, the present results indicate an association between the birth weight of siblings and the density of the serotonergic innervation in the frontal cortex at adulthood. This observation is difficult to compare with earlier findings of Minkowski and coworkers (1981) showing that intrauterine growth retardation resulted in elevated levels of monoamines and serotonin turnover in the forebrain immediately after birth. These authors used experimentally induced ametrohemia in the 5$^{th}$ day before delivery to induce this growth retardation. In contrast, in the present study the effects of naturally occurring variations of birth weights on the density of 5-HT transporters at adulthood were investigated.

It can not be excluded so far that prenatal factors, such as lower intrauterine blood supply to the smaller pups or differences in the intrauterine hormonal environment due to the sex of neighboring fetuses may have affected the development of 5-HT projections already during intrauterine life. However, it seems more likely that the association between birth weight and 5-HT innervation in the frontal cortex at adulthood is due to postnatal factors. The frontal cortex is characterized by a prolonged postnatal development (Dawirs et al., 1993; Moll et al., 2000) with lasting prominent remodeling processes during adolescence (Adams et al., 2000; Neddens et al., 2001; Winterfeld et al., 1998), whereas the monoaminergic innervation in the brainstem matures relatively early during the embryonic period of both rats (10th–16th days of gestation) and humans (3rd–7.5th weeks of gestation) (Rice and Barone, 2000). Since the different birth weights were associated with the development of the density of 5-HT transporters in the frontal cortex but not in the brain stem, the different size of the newborn rats may have affected the postnatal development of the frontal cortex (different rearing conditions), and therefore the development of its 5-HT innervation pattern.

Persisting alterations in serotonergic innervation patterns have been observed following neuro-psychopharmacological or neuro-toxic treatments during early postnatal live (Broening et al., 1995; Raines et al., 2001; Tonge, 1973), or by a disturbed energy or oxygen supply (Dell'Anna et al., 1993) or early psychopharmacological treatments (Wegerer et al., 1999). Also, acute psychological stress is known to increase serotonin turnover in many brain regions especially in prefrontal cortex (Inoue et al., 1994). Chronic stress, however, leading to behavioral deficits

of learned helplessness was found to be associated with reduced *in vivo* release of serotonin in the frontal cortex (Petty et al., 1992). Helpless rats showed decreased density of serotonin transporter sites in the medial part of prefrontal cortex as compared to controls (Wu et al., 1999). Alterations of the different types of 5-HT receptors were observed in different brain regions depending on the type of stressor (McKittrick et al., 1995; Watanabe et al., 1993). Although stress can have such profound affects on the serotonergic system of adult rats, only few reports show that psychosocial factors may alter serotonergic development early in life. Early physical stress to infant rats by chronic injection caused a decreased hippocampal 5HT1B-receptor mRNA expression, associated with a higher preference to alcohol during adolescence (Vazquez et al., 2002) and early psychological stress by maternal deprivation during infancy produced increased 5HT1A- and 5HT1B-receptor mRNA levels in the hippocampus. Also social isolation during the post-weaning period has been reported to produce persistent impairments of presynaptic serotonergic function in the frontal cortex and the hippocampus (Bickerdicke et al., 1993) and hypersensitivity to serotonergic stimulation in the nucleus accumbens (Lapiz et al., 2003). Most interestingly, long lasting early social deprivation was associated with an increased density of 5HT-positive fibers in distinct sub-regions of the prefrontal cortex (Poeggel et al., 2003), hippocampus (Busche et al., 2002), and both insular and entorhinal cortices (Neddens et al., 2003). The general conclusion of these results is that the development of 5-HT projections appears to be very vulnerable to modulations caused by various chemical, physical and psychosocial environmental variables. Furthermore, clinical implications of the serotonergic system have been widely discussed recently (cf. Sodhi and Sanders-Bush, 2004; Whitacker-Azmitia, 2001; Mann, 1999).

Remarkably, we did not detect any significant effects of high or low birth weight on the noradrenergic afferences to the frontal cortex. Whereas the serotonergic system is rather involved in regulating cognitive and emotional processes, the noradrenergic system is an important mediator of arousal and acute stress responses (Carrasco and Van der Kar, 2003). Acute stressors stimulate the firing rate of neurons in the locus ceruleus and the release of noradrenalin throughout the forebrain (Levine et al., 1990). Chronic stress is associated with elevated release of noradrenalin upon exposure to subsequent stressors (Nisenbaum et al., 1991) and enhanced transcription of the tyrosine hydroxylase gene in the locus ceruleus (Rusnak et al., 2001). Early stress has been shown to cause a lifelong increased sensitivity of the noradrenergic system (Liu et al., 2000). However, there is no published evidence that permanent alterations of noradrenergic innervation patterns in the frontal cortex may be caused by psychosocial variables.

In conclusion, the present data have shown an association between body weight of littermates at birth and subsequent development of the serotonergic system. The development of serotonergic projections into the FC appears to be especially vulnerable to factors or conditions associated with initial differences of birth weight, e.g. different experiences of maternal care during early life. Therefore, low birth weight may be a risk factor for the development of the serotonergic system.

## References

Adams J, Barone S Jr, LaMantia A, Philen R, Rice DC, Spear L, Susser E (2000) Workshop to identify critical windows of exposure for children's health: neurobehavioral work group summary. Environ Health Perspect 108(Suppl 3): 535–544

Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ (2002) Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. JAMA 288: 728–737

Bickerdicke MJ, Wright LK, Marsden CA (1993) Social isolation attenuates rat forebrain 5HT release induced by KCl stimulation and exposure to a novel environment. Behav Pharmacol 4: 231–236

Broening HW, Bowyer JF, Slikker W Jr (1995) Age-dependent sensitivity of rats to the long-term effects of the serotonergic neurotoxicant (±) 3,4-methylenedioxymethamphetamine (MDMA) correlates with the magnitude of the MDMA-induced thermal response. J Pharmacol Exp Ther 27: 325–333

Busche A, Neddens J, Dinter C, Dawirs RR, Teuchert-Noodt G (2002) Differential influence of rearing conditions and methamphetamine on serotonin fibre maturation in the dentate gyrus of gerbils (Meriones unguiculatus). Dev Neurosci 24: 512–521

Carrasco GA, Van de Kar LD (2003) Neuroendocrine pharmacology of stress. Eur J Pharmacol 463: 235–272

Dawirs RR, Teuchert-Noodt G, Czaniera R (1993) Maturation of the dopamine innervation during postnatal development of the prefrontal cortex in gerbils (Meriones unguiculatus). A quantitative immunocytochemical study. J Hirnforsch 34: 281–291

Dean B (2003) The cortical serotonin2A receptor and the pathology of schizophrenia: a likely accomplice. J Neurochem 85: 1–13

Dell'Anna ME, Luthman J, Lindqvist E, Olson L (1993) Development of monoamine systems after neonatal anoxia in rats. Brain Res Bull 32: 159–170

Gunnell D, Rasmussen F, Fouskakis D, Tynelius P, Harrison G (2003) Patterns of fetal and childhood growth and the development of psychosis in young males: a cohort study. Am J Epidem 158: 291–300

Hawi Z, Dring M, Kirley A, Foley D, Kent L, Craddock N, Asherson P, Curran S, Gould A, Richards S, Lawson D, Pay H, Turic D, Langley K, Owen M, O'Donovan M, Thapar A, Fitzgerald M, Gill M (2002) Serotonergic system and attention deficit hyperactivity disorder (ADHD): a potential susceptibility locus at the 5-HT(1B) receptor gene in 273 nuclear families from a multi-center sample. Mol Psych 7: 718–725

Hoffman BJ, Hansson SR, Mezeya E, Palkovits M (1998) Localization and dynamic regulation of biogenic amine transporters in the mammalian central nervous system. Front Neuroendocrinol 19: 187–231

Hultman CM, Sparen P, Takei N, Murray RM, Cnattingius S (1999) Prenatal and perinatal risk factors for schizophrenia, affective psychosis, and reactive psychosis of early onset: case control study. BMJ 318: 421–426

Inoue T, Tsuchiya K, Koyama T (1994) Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. Pharmacol Biochem Behav 49: 911–920

Kent L, Doerry U, Hardy E, Parmar R, Gingell K, Hawi Z, Kirley A, Lowe N, Fitzgerald M, Gill M, Craddock N (2002) Evidence that variation at the serotonin transporter gene influences susceptibility to attention deficit hyperactivity disorder (ADHD): analysis and pooled analysis. Mol Psych 7: 908–912

Lapiz MD, Fulford A, Muchimapura S, Mason R, Parker T, Marsden CA (2003) Influence of post weaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neurosci Behav Physiol 33: 13–29

Levine ES, Litto WJ, Jacobs BL (1990) Activity of cat locus ceruleus noradrenergic neurons during the defense reaction. Brain Res 531: 189–195

Liu D, Caldji C, Sharma S, Poltsky PM, Meaney MJ (2000) Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinephrine release in the hypothalamic paraventricular nucleus. Neuroendocrinol 12: 5–12

Lowry OH, Rosebourgh NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Mann JJ (1999) Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. Neuropsychopharmacology 21: 99S–105S

Matsumoto H, Takei N, Saito F, Kachi K, Mori N (2001) The association between obstetric complications and childhood-onset schizophrenia: a replication study. Psychol Med 31: 907–914

McKittrick CR, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR (1995) Serotonin receptor binding in a colony model of chronic social stress. Biol Psychiatry 37: 383–393

Mick E, Biedermann J, Princ J, Fischer MJ, Faraone SV (2002) Impact of low birth weight on attention-deficit hyperactivity disorder. J Dev Behav Ped 23: 16–22

Minkowski A, Chanez C, Priam M, Flexor MA, Hamon M, Bourgoin S, Kordon C (1981) Long lasting effects of intrauterine malnutrition on neurotransmitters metabolism in the brain of developing rats. Prog Clin Biol Res 77: 643–660

Moll GH, Hause S, Ruther E, Rothenberger A, Huether G (2001) Early methylphenidate administration to young rats causes a persistent reduction in the density of striatal dopamine transporters. J Child Adolesc Psychopharmacol 11: 15–24

Moll GH, Mehnert C, Wicker M, Bock N, Rothenberger A, Ruether E, Huether G (2000) Age-associated changes in the densities of presynaptic monoamine

transporters in different regions of the rat brain from early juvenile life to late adulthood. Dev Brain Res 119: 251–257

Neddens J, Bagorda F, Busche A, Horstmann S, Moll GH, Dawirs RR, Teuchert-Noodt G (2003) Epigenetic factors differentially influence postnatal maturation of serotonin (5-HT) innervation in cerebral cortex of Gerbils: interaction of rearing conditions and early methamphetamine challenge. Dev Brain Res 146: 119–130

Neddens J, Brandenburg K, Teuchert-Noodt G, Dawirs RR (2001) Differential environment alters ontogeny of dopamine innervation of the orbital prefrontal cortex in gerbils. J Neurosci Res 63: 209–213

Nisenbaum LK, Zigmond MJ, Sved AF, Abercrombie ED (1991) Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. J Neurosci 11: 1478–1484

Olness K (2003) Effects on brain development leading to cognitive impairment: a world wide epidemic. J Dev Behav Ped 24: 120–130

Petty F, Kramer G, Wilson L (1992) Prevention of learned helplessness: in vivo correlation with cortical serotonin. Pharmacol Biochem Behav 43: 361–367

Poeggel G, Nowicki L, Braun K (2003) Early social deprivation alters monoaminergic afferents in the orbital prefrontal cortex of Octodon degus. Neuroscience 116: 617–620

Powers RE (1999) The neuropathology of schizophrenia. J Neuropathol Exp Neurol 58: 679–690

Raines KW, Seidler FJ, Slotkin TA (2001) Alterations in serotonin transporter expression in brain regions of rats exposed neonatally to chlorpyrifos. Dev Brain Res 130: 65–72

Rice D, Barone S (2000) Critical periods of vulnerability for the developing nervous system: evidence from human and animal models. Environ Health Perspect 108(Suppl 3): 511–533

Rusnak M, Kvetnansky R, Jelokova J, Palkovits M (2001) Effects of novel stressors on gene expression of tyrosine hydroxylase and monoamine transporters in brainstem noradrenergic neurons of long-term repeatedly immobilized rats. Brain Res 899: 20–35

Saigal S, Pinelli J, Hoult L, Kimm MM, Boyle M (2003) Psychopathology and social competencies of adolescents who were extremely low birth weight. Pediatrics 111: 969–975

Sawa A, Snyder SH (2002) Schizophrenia: diverse approaches to a complex disease. Science 296: 692–695

Sodhi MS, Saunders-Bush E (2004) Serotonin and brain development. Int Rev Neurobiol 59: 111–174

Thompson C, Syddall H, Rodin L, Osmond C, Barker DJ (2001) Birth weight and the risk of depressive disorder in late life. Br J Psych 179: 450–455

Tonge S (1973) Permanent alterations in 5-hydroxyindole concentrations in discrete areas of rat brain produced by the pre- and neonatal administration of methylamphetamine and chlorpromazine. J Neurochem 20: 625–627

Vazquez DM, Eskandari R, Zimmer CA, Levine S, Lopez JF (2002) Brain 5-HT receptor system in the stressed infant rat: implications for vulnerability to substance abuse. Psychoneuroendocrinology 27: 245–272

Watanabe Y, Sakai RR, McEwen BS, Mendelson S (1993) Stress and antidepressant effects on hippocampal and cortical 5-HT1A and 5-HT2 receptors and transporter sites for serotonin. Brain Res 615: 87–94

Wegerer V, Moll GH, Bagli M, Rothenberger A, Ruther E, Huether G (1999) Persistently increased density of serotonin transporters in the frontal cortex of rats treated with fluoxetine during early juvenile life. J Child Adolesc Psychopharmacol 9: 13–24

Whitacker-Azmitia PM (2001) Serotonin and brain development: role in human developmental disease. Brain Res Bull 56: 479–485

Winterfeld KT, Teuchert-Noodt G, Dawirs RR (1998) Social environment alters both ontogeny of dopamine innervation of the medial prefrontal cortex and maturation of working memory in gerbils (Meriones unguiculatus). J Neurosci Res 52: 201–209

Wu J, Kramer GL, Kram M, Steciuk M, Crawford IL, Petty F (1999) Serotonin and learned helplessness: a regional study of 5-HT1A, 5-HT2A receptors and the serotonin transport site in rat brain. J Psychiatr Res 33: 17–22

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