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Hyperactivity and alteration of the midbrain dopaminergic system in maternally stressed male mice offspring

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Abstract

We recently demonstrated that prolonged maternal stress produces profound and long-lasting deficits in brain functions by programming a subset of target genes. We have now examined the possible effects of prenatal stress on the motility of adult offspring and dopamine (DA)-related gene expression in their midbrains, one of the target brain regions of stress hormones. Maternally stressed adult male mice showed impaired response habituation to novelty, and increased wheel-running activity associated with altered responses to DA receptor and DA transporter (DAT) blockers. Along with the behavioral changes, the expression profiles of several genes of the midbrain DAergic system appeared to be altered. Expression of DAT was reduced and expression of DA receptors and striatal DA-regulated neuropeptide genes was also affected. Taken together, the present findings indicate that maternal stress can cause hyperactivity in adult offspring associated with alterations in the midbrain DAergic system suggestive of a functional hyperdopaminergic state.

Keywords: Maternal stress; Hyperactivity; Dopamine (DA); Dopamine transporter (DAT); Attention-deficit hyperactivity disorder (ADHD)

The environment in the womb can impact on the later life of the fetus. It is well established that low birth weight, for example, seems to be strongly correlated with increased risk of cardiovascular and metabolic disorders. To account for such long-lasting effects of environmental factors including prenatal stress, the concept of early life 'programming' has been proposed [1]. We have recently shown that prolonged maternal stress can have profound effects on the functions of various brain regions, in particular, the limbic system including hypothalamus, hippocampus, and amygdala [2,3]. In this regard it is noteworthy that prenatal stress can increase locomotor responses to novelty, and prenatal treatment with corticosterone can affect spontaneous and DA agonist-induced motor activity in rats [4,5]. Furthermore, alteration of catecholamine metabolism in discrete brain regions is also reported in maternally stressed adult offspring [4,6]. These observations raise the possibility that the midbrain dopaminergic (DAergic) system is a target of the programming effects of maternal stress, because dopamine (DA) is a neurotransmitter that is intimately involved in the control of motor activity as well as of cognition, emotion, and mood [7].

The brain regions that are most closely associated with hyperdopaminergia related to motor dysfunction are the caudate putamen and nucleus accumbens [7]. These are areas of the basal ganglia with a rich population of DA nerve terminals. Administration of DA agonists to these brain regions increases basal motor activity by activating DA receptors. By contrast, disruption of the nigrostriatal

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and mesolimbic DA system reduces both spontaneous locomotion and agonist-induced hyperactivity [5,8,9]. It has similarly been proposed that functional hyper or hypodopaminergia evoked by dysfunction of DA-related genes is responsible for altered motor activity. For instance, functional hyperdopaminergic states and subsequent increased locomotion are reported in dopamine transporter (DAT) knockout/knockdown mice [10,11]. DAT is believed to control the temporal and spatial activity of released DA by rapidly sequestering the neurotransmitter into pre-synaptic terminals to clear it from the synaptic cleft. In these mutant mice DA clearance is markedly impaired, and consequently extracellular DA is significantly elevated [10,11]. Their marked behavioral hyperactivity appears to almost entirely depend on DAergic transmission, since it is completely reversed by inhibition of tyrosine hydroxylase (TH) or blockade of DA receptors [12].

The present study was carried out to establish whether prolonged maternal stress induces hyperactivity in adult mice offspring, and to examine the possible modification of gene expression profiles in the midbrain DA system responsible for any behavioral alterations.

Materials and methods

Maternal stress procedure and tissue preparation. ICR mice from the Laboratory Animal Center at Seoul National University were used in all experiments and kept in temperature-controlled (22–23 °C) quarters under a 12-h light and dark photoperiod (light on at 7:00 a.m.); standard mouse chow and water were available *ad libitum*. The maternal stress procedure has been described previously [2,3]. Briefly, pregnant ICR mice were prepared by mating at the age of 6–7 weeks. Mice in the stress group were placed, individually, in a restrainer (a transparent plastic cylinder, 3 cm in diameter and 9 cm long) daily for 6 h (10:00 a.m. to 4:00 p.m.) from 8.5 days post coitum (dpc) to 18.5 or 19.5 dpc (the day before parturition). The control pregnant mice were undisturbed. The pups born to the stressed mice (STR) were weaned on postnatal day 21 (P21) and reared in an environment identical to that of the controls (CTL). Eight-week-old male offspring were used in all experiments. For tissue preparation, mice were killed between 11:00 a.m. and 2:00 p.m. and their brains were removed on ice. The substantia nigra-ventral tagmental area (SN-VTA) and striatum, which contain the caudate putamen and part of the nucleus accumbens, were dissected from 1-mm-thick brain slices prepared with a brain matrix and quickly frozen in liquid nitrogen. All animal procedures adhered to the Animal Care and Use Guidelines of Seoul National University.

Measurement of motor activity in response to novelty. The behavioral test for response habituation was performed during the light period (between 11:00 a.m. and 3:00 p.m.). Motor activity was measured for 60 min in a transparent activity-monitoring chamber containing multiple horizontal photocell arrays ($25 \times 25 \times 20$ cm chamber; infrared photobeams in a 16×16 array; San Diego Instruments, San Diego, CA). Naïve mice were kept in a room adjacent to the experimental room and exposed to the activity-monitoring apparatus only once during each test. Motility was expressed as mean beam-breaking latencies per 5 min period, or mean number of beam-breaks per min.

Recording of wheel-running activity. A subset of mice from each group was subjected to continuous monitoring of their wheel-running activity under a 12-h light/dark cycle (Mini Mitter Company, Bend, OR). After a period of adaptation exceeding 3 days, wheel-running activities were recorded and are presented as mean numbers of wheel-runs per 5 min. In the first experiment wheel-running activity was recorded over 2 days, and the results were divided into those in the light period and those in the dark period. In the second experiment wheel-running activity was recorded for only 6 h during the light period (10:00 a.m. to 4:00 p.m.) after the same period of time for habituation. Vehicle (physiological saline), haloperidol (a DA receptor antagonist; 10 mg/kg b.w.) or GBR12909 (a DAT blocker; 10 mg/kg b.w.) was administered subcutaneously 30 min prior to testing. The group means (±SE) of wheel-running activities were calculated from the individual mean activity of each mouse.

Plasmid preparation. cDNA fragments for Northern blot hybridization were amplified by RT-PCR using specific primers. PCR products were cloned into pGEM-T easy vector according to the manufacturer's instructions (Promega, Madison, WI) and confirmed by sequencing. Primer sequences used in cloning were as follows: DAT upper: 5′-CACTCTGGGTATCGACAGTG-3′; DAT lower: 5′-ATGGCATAGGCCAGTTTCTC-3′; TH upper: 5′-CATGTTGGCTGACCGCACAT-3′; TH lower: 5′-TAGCTAATGGCACTCAGTGC-3′; AADC upper: 5′-AGAAGAGGCAAGGAGATGGTGG-3′; AADC lower: 5′-AAGCGAAGAAATAGGGACTGTGC-3′; D1 upper: 5′-GACAACTGTGACACAAGGTTGAGC-3′; D1 lower: 5′-ATTACAGTCCTTGGAGATGGAGCC-3′; D2 upper: 5′-GCAGTCGAGCTTTCAGAGCC-3′; D2 lower: 5′-TCTGCGGCTCATCGTCTTAAG-3′; SP upper: 5′-TGAGCATCTTCTTCAGAGAATCGC-3′; SP lower: 5′-ATCGCTGGCAAACTTGTACAACTC-3′; PPD upper: 5′-ACTGCCATAGGGGGATTTGGTAGC-3′; PPD lower: 5′-CATAACATTAGAGGGCATCACGAG-3′; PPA upper: 5′-AACAGGATGAGAGC CACTTGC-3′; PPA lower: 5′-CTTCATC CGAGGGTAGAGACT-3′.

Northern blot hybridization. Total RNA preparation and Northern blot hybridization were performed as described previously with slight modifications [13]. Briefly, total RNAs from the SN-VTA and ST were isolated by the single-step acid guanidinium thiocyanate–phenol–chloroform method. Twenty micrograms of each RNA sample was resolved on a 1% formaldehyde agarose gel and transferred by diffusion blotting for 16 h to a Nytran filter (pore size: 0.45 μm, Schleicher & Schuell, Dachen, Germany). α-^{32}P-dCTP cDNA probes for each gene were generated by random priming. Hybridization was performed at 42 °C for 16 h in hybridization solution (50% deionized formamide, 5× SSPE, 5× Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured salmon-sperm DNA). After washing out unbound probe, the membranes were autoradiographed at −70 °C for 3 days.

Antibodies and immunoblot analysis. Anti-DAT (Chemicon, Temecula, CA) and TH (Sigma, St. Louis, MO) antibodies were obtained commercially. Whole cell extracts of the SN-VTA and ST were resolved by 10% SDS–PAGE and transferred to a PVDF membrane (Millipore, Bedford, MA) using Towbin's buffer (25 mM Tris, pH 8.3, 192 mM glycine, and 20% methanol). The blots were blocked in Tris-buffered saline (TBS; 150 mM NaCl, 10 mM Tris, pH 7.6, and 2 mM $MgCl_2$) containing 0.5% Tween 20 and 2% gelatin, and incubated with primary antibody at room temperature for 1 h. The blots were then washed four times with TBS/0.5% Tween 20 and incubated with secondary antibody linked to horseradish peroxidase (Jackson ImmunoResearch Laboratories, West Grove, PA). They were again washed four times, and immunoreactive bands were visualized with ECL reagents (Amersham Bioscience, Uppsala, Sweden).

Determination of striatal contents of DA and its metabolites. DA and its metabolites were measured by high-performance liquid chromatography coupled to an electrochemical detector (HPLC-ECD) system (Gilson, Middletown, WI) as described previously with modifications [13]. Striatal fragments were homogenized in 0.1 N perchloric acid (0.6 ml/striatal fragment) containing 40 mM sodium metabisulfite and centrifuged at 10,000*g*. The supernatants were recovered, microfiltered, and applied. The striatal contents of DA and its metabolites were normalized with soluble protein content and expressed in pg/μg protein.

Data analysis. Relative expression levels from the Northern blot hybridization or immunoblot analyses were determined by densitometry. The data from the behavior tests, Northern blotting, and immunoblotting were evaluated statistically with Student's *t*-test. A probability level of $p < 0.05$ was taken as statistically significant.

Results

To test whether prolonged maternal stress affects the motor activity of adult mice offspring, we adopted two experimental paradigms. These were response habituation in a novel environment and wheel-running activity in the home cage, both of which are known to depend on the midbrain DAergic system [10,11,14]. In the first experiment, measuring motility in a novel environment, both groups of mice had similar initial beam-breaking latencies. However, in the control mice these increased significantly with habituation (after 40 min) in the testing apparatus whereas they increased only marginally in the maternally stressed mice, and there was significant hyperactivity compared to the control mice during the last 20 min (Fig. 1A). Similarly, the mean numbers of beam-breaks of the maternally stressed mice were essentially the same as those of the controls during the first half of the testing period. But during the last 30 min the maternally stressed mice appeared hyperactive by virtue of a larger mean number (approximately 3-fold more) of beam-breaks than those of the controls (Fig. 1B), confirming their hyperactivity and impaired motor response habituation to novelty. The maternally stressed mice were also hyperactive in wheel-running behavior in their home cage, particularly during the daytime (Fig. 1C). During the night corresponding to the activity period of mice, there was only a slight increment of wheel-running activity in the stressed mice. The hyperactive wheel-running in the maternally stressed mice was presumably due to a hyperdopaminergic state, since it disappeared in response to systemic administration of haloperidol, a DA receptor antagonist, and the motilities of both treated groups were comparable to that of the vehicle-treated control mice. Moreover indirect induction of a hyperdopaminergic state with GBR12909, a DAT blocker, increased wheel-running activity in the control mice but not in the maternally stressed mice (Fig. 1D). These results strongly suggest that alterations in the DAergic system contribute to the hyperactivity of the maternally stressed adult male mice.

The midbrain DAergic systems, particularly the nigrostriatal and mesolimbic DAergic systems, have been implicated in regulation of motor activities [7]. We therefore examined the mRNA and protein levels of several DA-related genes such as DAT, TH, and aromatic L-amino acid decarboxylase (AADC) in two discrete brain regions: the ventral midbrain region containing the substantia nigra and ventral tagmental area (SN-VTA), in which a large portion of the DAergic cell bodies are known to reside, and the striatum (ST) containing the caudate putamen

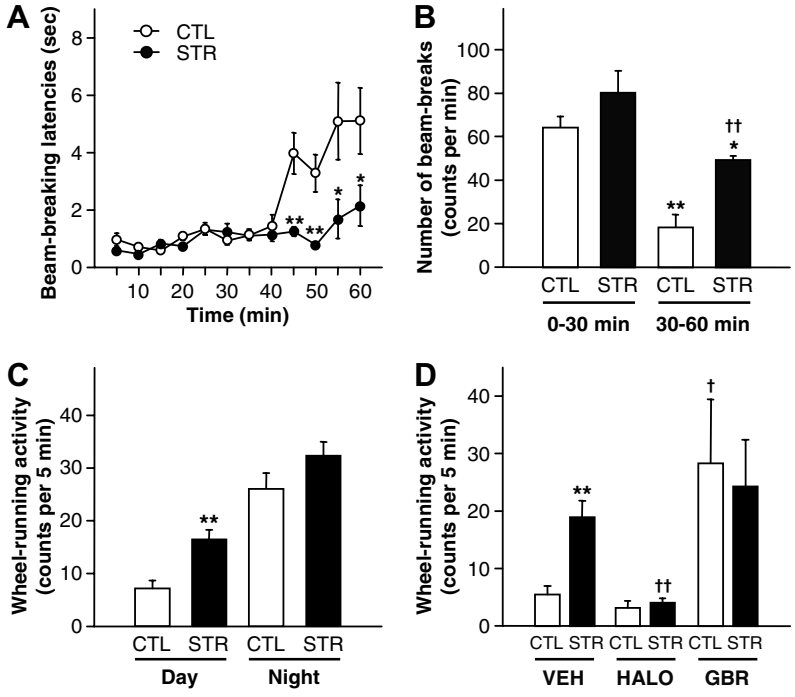

Fig. 1. Impaired response habituation to novelty and hyperactivity of maternally stressed adult offspring. (A) Motor activities of naïve control and maternally stressed mice were examined for 60 min in an activity-monitoring chamber and are presented as means ± SE of beam-breaking latencies ($^{*}p < 0.05$ and $^{**}p < 0.01$ vs. control mice; $n = 6$). (B) Mean numbers of beam-breaks are presented as mean counts per min ± SE ($^{**}p < 0.01$ vs. 0–30 min control mice; $^{*}p < 0.05$ vs. 0–30 min stressed mice; $^{\dagger\dagger}p < 0.01$ vs. 30–60 min control mice; $n = 6$). (C) Wheel-running activities of mice of each group were recorded for 48 h. Mean activities during the light (Day) and dark (Night) periods were calculated separately and are presented as means ± SE of number of wheel-runs in 5 min ($^{**}p < 0.01$ vs. control; $n = 5$). (D) Vehicle (physiological saline), haloperidol (HALO; DA receptor antagonist; 10 mg/kg b.w.) or GBR12909 (GBR; DAT blocker; 10 mg/kg b.w.) was administered subcutaneously 30 min prior to the recording period, and wheel-running was recorded for 6 h during the light period (10:00 a.m. to 4:00 p.m.). Means ± SE number of wheel-runs per 5 min are shown ($^{**}p < 0.01$ vs. vehicle-treated control mice; $^{\dagger}p < 0.05$ vs. vehicle-treated control mice; $^{\dagger\dagger}p < 0.01$ vs. vehicle-treated stressed mice; $n = 4$ for each group).

and nucleus accumbens as major target regions of the projections from the midbrain DAergic neurons. Transcripts of these genes were only detected in the SN-VTA, but the DAT and TH proteins were found in both the SN-VTA and ST, as expected. Expression of TH and AADC was not significantly altered in these brain regions of the maternally stressed mice. However, the mRNA and protein levels of DAT in the maternally stressed mice were only about half of those in the controls (Fig. 2A and B).

To examine the functional relevance of the reduced level of DAT, we carried out an HPLC-coupled electrochemical analysis of the contents of DA and its metabolites in the striatal tissues. Levels of DA were not significantly changed in the stressed mice (Fig. 2C), in agreement with the unaltered expression of TH. In spite of this, the ratios of the DA metabolites, DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanilic acid), to DA were significantly elevated in the striatal tissues of the maternally stressed mice, pointing to enhanced DA turnover (Fig. 2D).

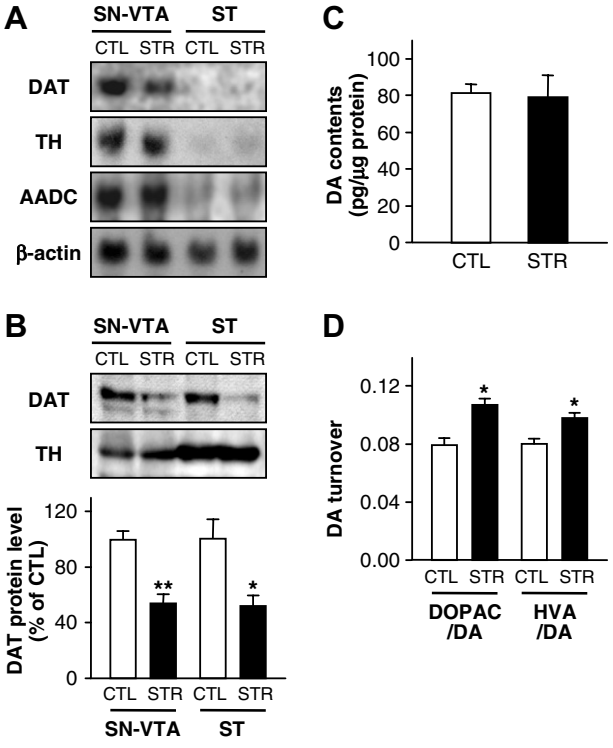

Fig. 2. Reduced midbrain DAT expression and increased DA turnover in the striatum of maternally stressed mice. The substantia nigra and ventral tagmental area (SN-VTA) and the striatal regions (ST) were isolated from control and maternally stressed mice. (A) Northern blot hybridization was performed using ^{32}P-labeled cDNA probes complementary to the mouse dopamine transporter (DAT), tyrosine hydroxylase (TH), and aromatic L-amino acid decarboxylase (AADC) mRNAs. Blots were re-probed with a ^{32}P-labeled cDNA probe for β-actin as an internal control. Representative blots are presented. (B) Lysates of the SN-VTA and ST were prepared and immunoblot analyses were performed with anti-DAT and TH antibodies. Representative blots are shown in the upper panel. The relative amounts of DAT proteins were determined by densitometric analyses and are shown in the lower panel ($^{*}p < 0.05$ and $^{**}p < 0.01$ vs. control; $n = 6$). (C) Striatal DA levels were determined by HPLC-ECD and are shown as means ± SE ($n = 6$). The measured levels were normalized with the amount of soluble protein in each homogenate. (D) The DA turnover rate was calculated from the ratio of DA metabolites such as DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanilic acid) to DA, and are presented as means ± SE ($^{*}p < 0.05$ vs. control mice; $n = 6$).

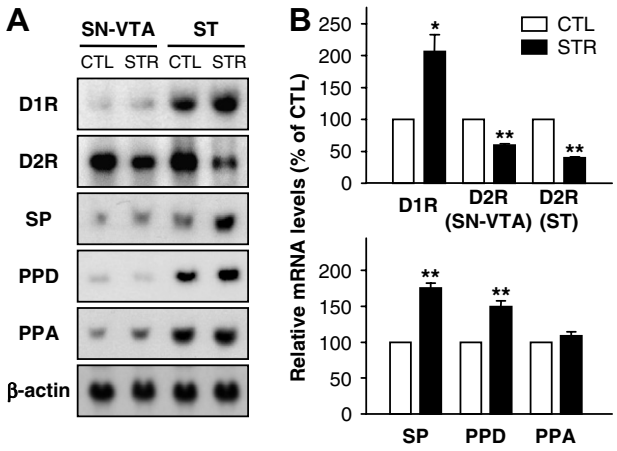

Fig. 3. Expression of DA receptors and striatal neuropeptides. (A) Representative blots for the D1 DA receptor (D1R), D2 DA receptor (D2R), substance P (SP), preprodynorphin (PPD), and preproenkephalin A (PPA). (B) Relative transcript levels were determined by densitometric analyses, normalized with β-actin, and expressed as means ± SE% of the control mice ($^{*}p < 0.05$ and $^{**}p < 0.01$ vs. control by paired Student's t-test; $n = 4$).

In the next experiments we examined maternal stress-evoked alterations in the transcripts of the DA receptors and striatal neuropeptides by Northern blot hybridization. The D1 subtype of DA receptor mRNA was detected only in the ST and its expression increased approximately 2-fold in the maternally stressed mice (207.7 ± 26.6% of the control mice, $p < 0.05$); in contrast, D2 receptor mRNA was detected in the SN-VTA and ST, and its expression was reduced in both regions (59.9 ± 1.7% of control in the SN-VTA; 39.9 ± 1.5% in the ST; Fig. 3). The expression of several neuropeptide precursor genes such as substance P (SP), preprodynorphin (PPD), and preproenkephalin A (PPA) in the ST is regulated by DA [15,16]. The neurons producing each of these genes display characteristic subtypes of DA receptor. Transcription of SP and PPD mRNAs is known to be induced by DA via D1 receptors, while PPA transcription is inhibited by activation of D2 receptors. To confirm the alterations in DAergic transmission in the ST, we examined changes in the expression of these neuropeptide genes. SP and PPD mRNAs were significantly elevated in the ST of the maternally stressed mice (SP: 175.7 ± 6.8% of control mice, $p < 0.01$; PPD: 149.9 ± 8.2% of control mice, $p < 0.01$), with no significant difference in PPA mRNA between the two groups (Fig. 3).

Discussion

The present study was undertaken to determine whether prolonged maternal stress influences motor behavior and

the midbrain DAergic system of adult mice offspring by modulating relevant gene expression. We employed two behavioral paradigms: motor responses to a novel environment and wheel-running activities in the home cage. In combination with these behavioral experiments, analysis of gene expression revealed, for the first time, that DAT and DA receptors are molecular targets implicated in the maternal stress-induced hyperactivity in the stressed offspring.

Although long-lasting influences of prenatal exposure to stress or stress hormones on the motor activities of the adult offspring have been established, some of the findings in rats are controversial. Some workers report that maternal stress or prenatal exposure to excess glucocorticoid results in increased spontaneous locomotor activity, or more active motor behavior in response to a novel situation with shorter latencies of exploration [4–6]. In contrast, others claim that prenatally stressed rats show decreased exploration or locomotor activity in a novel situation [17,18]. It is likely that these conflicting results are due to affects on fear and anxiety. In fact, these can significantly distort motor responses in a novel environment. The suppression of motor activity in an open field has even been used as an index of fear and anxiety in rodents. However, the maternally stressed male mice in the present study underwent no significant changes in anxiety state as assessed by the elevated plus-maze test [2]. More importantly, they showed increased wheel-running activity during the daytime even after more than 3 days of habituation. Collectively, these results indicate that prolonged maternal stress has a tendency to induce elevated motor activity in the adult mice offspring.

As shown in Fig. 1D, increased motor activity in the maternally stressed mice was apparently dependent on the DAergic system. Administration of a DA receptor antagonist reduced the wheel-running activity of stressed mice to a level comparable with control mice. The midbrain DAergic system, particularly the nigrostriatal and mesolimbic pathways, is most closely associated with the regulation of motor activity, since administration of a DA agonist to the caudate putamen or the nucleus accumbens, as well as systemic administration, increases spontaneous motor activity by activating DA receptors [7,10] and agonist-induced hyperactivity is abolished by disrupting the DA system [5,8,9]. The present study coupled with these previous findings strongly suggest that the alterations in the midbrain DAergic system could evoke the hyperdopaminergic state in the maternally stressed mice. Interestingly, the maternally stressed mice had reduced sensitivity to indirect increments of DAergic tone resulting from treatment with GBR12909, a DAT blocker, pointing to a possible defect in DAT function and/or an altered DA receptor system. This result is consistent with their reduced levels of DAT protein in both the SN-VTA and ST regions. DAT is believed to contribute to the termination of DAergic transmission by clearing the DA released into the synaptic cleft. Inhibition of DAT clearly increases extracellular DA and induces hyperlocomotor activity [19]. More importantly, functional hyperdopaminergia evoked in DAT knockout/knockdown mice results in impaired response habituation and marked hyperactivity coupled with elevated extracellular DA levels in the ST [10,11]. It is also of interest that, as in DAT knockdown mice [11], DA turnover, or tissue content of DOPAC and HVA relative to DA, in the striatal regions is augmented in the stressed mice. This result is consistent with other studies showing that prenatal exposure to excess GC can alter DA metabolism in various brain regions including the ST of adult offspring [4,6]. The increased DA turnover probably reflects an elevation of extracellular DA in the stressed offspring in view of the facts that increased DA turnover was found to accompany elevated extracellular DA in both genetic and pharmacological studies [11,20], and that the levels of the monoamine oxidases and cathechol-*O*-methyl transferase responsible for DA turnover are not significantly increased by maternal stress (data not shown).

In addition to DAT, expression of DA receptors in the ventral midbrain and striatal regions of adult offspring is also influenced by maternal stress. In the ST of the maternally stressed mice, expression of the D1 receptor was augmented, whereas that of the D2 receptor was reduced. Only D2 receptor mRNA was abundant in the SN-VTA region, and its level was also lower in the stressed animals. The distribution of these receptors is consistent with the previous finding that D1 receptors are mainly post-synaptic whereas D2 receptors are both pre- and post-synaptic [21]. Several previous studies have shown that increased DAergic transmission down-regulates both D1 and D2 receptors in the ST [10,22]. However, down-regulation of mouse D1 receptor mRNA by DA-deficiency, and differential regulation of the expression of the DA receptors by their ligand depending on developmental state, have also been demonstrated [23–25]. Therefore, it is unlikely that the differential expression of D1 and D2 receptors is simply due to the increased DAergic transmission in maternally stressed mice. It should be also noted that the D1 and D2 receptors appear to be largely expressed in restricted populations of neurons. For instance, SP or PPD-expressing neurons contain mainly D1 receptor, whereas PPA-positive neurons contain the D2 receptor. As a result of this restricted expression SP and PPD are found to be controlled by stimulation by DA whereas PPA expression is negatively controlled via the D2 receptor [15,16]. Thus, expression of the neuropeptides can be used as indices of DAergic transmission. As shown in Fig. 3, SP and PPD mRNAs were augmented in the ST of maternally stressed mice, but PPA mRNA was unchanged. This result, in combination with the DA receptor mRNA levels, strongly suggests that D1 receptor-mediated DAergic transmission is particularly enhanced in the stressed mice. Interestingly, the differential regulation of the mRNAs for the striatal peptides in the present study is comparable with that in animals exposed to chronic administration of amphetamine, which is known to induce a hyperdopaminergic state by increasing the release of DA

into the synaptic cleft [25]. In the light of the finding that administration of a D1 receptor-specific agonist is sufficient to induce hyperlocomotion, whereas a D2-specific agonist fails to do [26], the increased motor activities in the stressed mice seem to be primarily due to increased D1-mediated DAergic transmission. In addition, there is also considerable evidence that induction of SP contributes significantly to the control of motor behaviors by DA. For example, SP is reported to increase striatal DA release, and blockade of SP receptors decreases both amphetamine-induced behavioral activity and the induction of these striatal DA-regulated neuropeptides [27,28].

There is evidence that DA has a crucial role in regulating the motor activity associated with attention-deficit hyperactivity disorder (ADHD), a human neurological disorder characterized by hyperactivity, impulsivity, and poor sustained attention [29]. Brain imaging suggests that children with ADHD have a midbrain DAergic dysfunction at the level of the DAergic nucleus [30] and genetic studies also provide evidence that genes involved in the regulation of nigrostriatal and mesolimbic DAergic transmission are responsible for the onset of ADHD. Dysfunction of the DAT, one type of DA receptor, and the DA-beta-hydroxylase (DβH), is strongly correlated with ADHD [29,31]. In this context, it is of importance that the DAT knockout/knockdown mice display several of the key characteristics of individuals with ADHD, such as hyperdopaminergia-evoked hyperactivity, cognitive impairment, and paradoxical calming responses to psychostimulants, supporting the importance of DAT in the onset and severity of ADHD [10–12,32]. It is well established that both genetic and environmental factors are associated with the severity and maintenance of ADHD [29]. In particular, studies on human populations have suggested a possible correlation between stress during pregnancy and the onset of ADHD. One case-control study showed that mothers of children with ADHD more frequently reported psychosocial stress during pregnancy [33] and another study indicated that children whose mothers were exposed to stressful circumstances during pregnancy had a higher incidence of inattention problems [34].

The present findings demonstrate that prolonged maternal stress can cause hyperactivity of the motor behaviors of their adult male mice offspring, associated with alterations in central DAergic gene expression, although the exact molecular mechanism responsible for reprogramming the gene expression requires clarification. In connection with the evidence for involvement of the hyperdopaminergic state in the onset of ADHD and the possible linkage between this disease and prenatal stress in human populations [33,34], our results strongly suggest that maternal stress may increase the risk of these neurological disorder by affecting the neurotransmitter system in such a way as to alter the expression of critical target genes such as DAT, DA receptors and SP.

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