Sleep deprivation differentially affects dopamine receptor subtypes in mouse striatum

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The effects of sleep deprivation on dopaminergic systems remain elusive, in part due to the lack of selective ligands for dopamine receptor subtypes. We examined D_1, D_2, and D_3 receptor density in the mouse brain after sleep deprivation by receptor autoradiography using [^{3}H]SCH 23390 for D_1R, [^{3}H]raclopride for D_2R, and [^{3}H]WC-10 for D_3R (a novel D_3R-selective compound developed in our laboratory, not previously reported in mouse). Sleep-deprived mice showed a significant decrease in D_1R, no change in D_2R, and a significant increase in D_3R binding in striatum. This pattern of dopamine receptor changes was not seen in mice subjected to restraint stress, suggesting specificity to sleep. These data provide evidence that brain dopaminergic circuits are remodeled after sleep deprivation. *NeuroReport* **22:489–493 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Multiple neurotransmitters are implicated in the sleep–wake cycle. There is recent evidence for the role of dopamine in the regulation of sleep and wakefulness. Microdialysis studies in rat have found that dopamine levels in the prefrontal cortex and nucleus accumbens exhibit a diurnal rhythm [1]. Mice lacking the dopamine transporter (DAT) gene show increased wakefulness, whereas mice lacking the dopamine D_2 receptor show significantly decreased wakefulness [2,3]. Medications used to maintain wakefulness increase dopaminergic activity by primarily targeting the DAT reuptake mechanism [4]. In addition, patients with Parkinson's disease, who show neurodegeneration of the striatonigral dopaminergic system, experience sleep disturbances including excessive daytime sleepiness [5]. Taken together, these studies suggest a probable role for dopamine in the maintenance of wakefulness.

The effects of sleep deprivation on dopamine activity have recently been studied in the human brain using PET radiotracers. Healthy human participants underwent one night of sleep deprivation and were imaged with [^{11}C]raclopride, which targets primarily D_2 receptors (D_2R) with less binding to D_3 receptors (D_3R) [6]. [^{11}C]raclopride binding was significantly reduced in the striatum and thalamus. Reduced binding suggests greater endogenous dopamine binding to D_2R/D_3R after sleep deprivation, possibly as a countermeasure to promote wakefulness during sleep deprivation [7]. However, the specific contribution of D_3R to sleep deprivation remains to be determined. Until this point it has been difficult to examine D_3R due to lack of selective pharmacologic tools.

Recently, a novel D_3R selective radioligand was developed in our laboratory, [^{3}H]WC-10, or [^{3}H]4-(dimethylamino)-N-[4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl]benzamide, a N-phenyl piperazine analog which displays high affinity and selectivity for D_3R [8–10]. The first quantitative autoradiographic analysis of the binding of [^{3}H]WC-10 was performed in rat and rhesus monkey brain confirming localization of D_3R to the striatum, among other brain regions [9].

The aim of this study is to characterize the effects of sleep deprivation on D_1R, D_2R, and D_3R binding in mouse striatum. As a comparison with sleep deprivation, we also examined the effects of restraint stress on D_1R, D_2R, and D_3R binding in mouse striatum. This is the first report of the use of the [^{3}H]WC-10 to localize D_3R binding in mouse.

Methods

Animals

All experimental procedures involving animals were performed in accordance with guidelines established by the Animal Studies Committee at Washington University in St Louis. All experiments were performed in a 12-h dark and 12-h light controlled room (lights on at 7:00 h and lights off at 19:00 h). Animals had access to food and water *ad libitum* throughout the experiments.

Sleep deprivation

C57BL6 female mice at 2 months of age ($n = 5$) were sleep-deprived for 72 h using the platform-over-water method as previously described [11]. In brief, mice were placed on platforms consisting of a closed PVC pipe measuring 3.4 cm in diameter, which was affixed to the

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bottom of a regular sized shoebox cage, with water filling the cage to 1 cm below the surface of the PVC pipe. This method has been validated by us and other laboratories with electroencephalography/electromyography electrode recording to substantially reduce both rapid eye movement (REM) and non-REM sleep [11]. Age-matched and sex-matched control mice ($n = 4$) remained isolated in their home cages during the 72 h. The 72 h time point was chosen based on previous methodology using the platform-over-water method to induce chronic sleep deprivation [12]. Mice were then sacrificed and their brains were immediately removed and fresh-frozen on powdered dry ice. Brains were sectioned at 30 μm on a cryostat and thaw-mounted onto Fisher Superfrost Plus slides, with 10 sets taken from the rostral through caudal striatum. Slides were stored at −80°C until processing for receptor autoradiography.

Restraint stress

Age-matched 2-month-old C57BL6 mice were randomly assigned to restraint stress or control groups ($n = 6$ per group). Mice were subjected to restraint stress in a 50 ml conical tube for 2 h daily for 7 days in total as previously described [13,14], with the time point chosen to represent chronic stress. Control mice remained in their home cages. On day 7, all mice were sacrificed. Brains were removed, snap-frozen on powdered dry ice, and sectioned using a cryostat as described above.

Precursor synthesis and radiolabeling

[^{3}H]WC-10 was synthesized by American Radiolabeled Chemicals (St Louis, Missouri, USA) by alkylation of the desmethyl precursor with [^{3}H]methyl iodide. The specific activity of the radioligand was 80 Ci/mmol. The detailed synthesis scheme for [^{3}H]WC-10 has been previously described [10].

Quantitative receptor autoradiography

All sections were preincubated for 20 min in buffer (50 mM Tris buffer, pH 7.4, 25°C, containing 120 mM NaCl, 5 mM KCl) to remove endogenous dopamine binding. After incubation with the respective tracer or nonspecific binding buffers, slides were then rinsed five times at 1 min intervals with ice-cold buffer. Slides were incubated in an open staining jar with the free radioligand concentration loss at less than 5%, as previously described [9,10].

D_1 receptor binding

D_1 receptors were labeled with [^{3}H]SCH 23390 using the procedure for rat brain described by Savasta *et al.* [15] with minor modifications. Specifically, brain sections were incubated for 15 min in a 7.4 pH buffer solution containing 50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, and 1 mM $MgCl_2$ at room temperature. Sections were then incubated for 60 min at room temperature in a similar buffer solution with the addition of 1.5 nM [^{3}H]SCH 23390 (PerkinElmer Inc., Waltham, Massachussetts, USA) and 30 nM ketanserin tartrate (Tocris Bioscience, Ellisville, Missouri, USA) to block 5-HT_2 receptors. Nonspecific binding was determined from the slides in the presence of 1 μM (+)-butaclamol, as described previously [9,16].

D_2 receptor binding

D_2 receptors were labeled with [^{3}H]raclopride using the previously described procedure for rat and monkey tissue [9]. Brain sections were incubated for 60 min in buffer solution at room temperature with the addition of 4 nM [^{3}H]raclopride (PerkinElmer). Nonspecific binding was determined from the slides in the presence of 1 μM s(−)-eticlopride, as before [9].

D_3 receptor binding

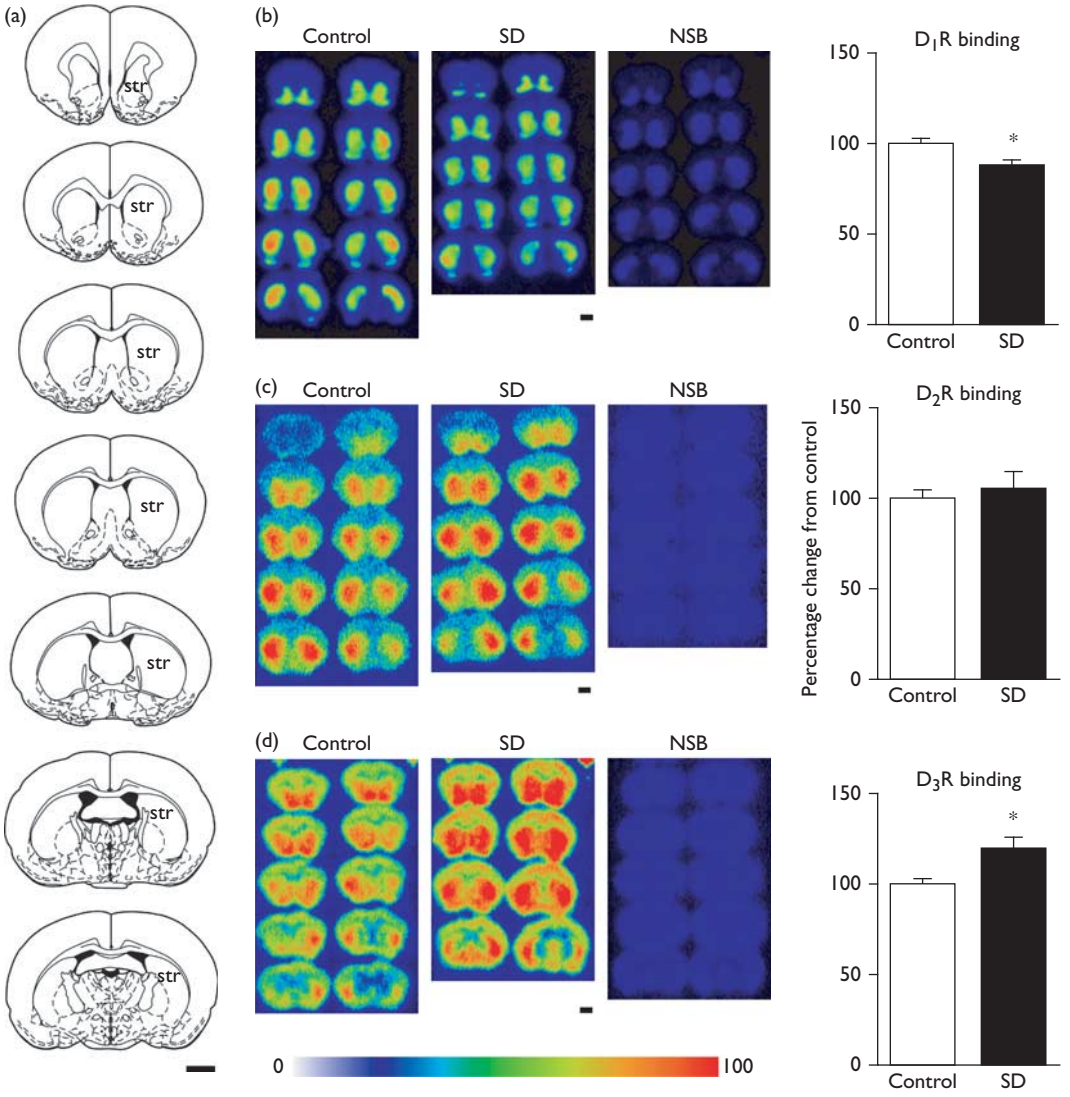
D_3 receptors were labeled with [^{3}H]WC-10 using the previously described procedure for rat and monkey tissue [9]. Brain sections were incubated for 60 min in buffer solution at room temperature with the addition of 4 nM [^{3}H]WC-10. Nonspecific binding was determined from the slides in the presence of 1 μM s(−)-eticlopride, as before [9].

Quantification of total radioactivity

Slides were air dried and made conductive by coating the free side with a copper foil tape. Slides were then placed into a the gas chamber containing a mixture of argon and triethylamine (Sigma-Aldrich, USA) as part of a gaseous detector system, the Beta Imager 2000Z Digital Beta Imaging System (Biospace, France). After the gas was well mixed and a homogenous state was reached, further exposure for 24 h yielded high-quality images. A [^{3}H]Microscale (American Radiolabeled Chemicals, St Louis, Missouri, USA) was counted simultaneously as a reference for total radioactivity quantitative analysis. Quantitative analysis was performed with the program Beta-Vision Plus (BioSpace, France) for the anatomical region of interest.

A total of 7–8 brain sections were chosen for each animal. Using known neuroanatomical markers, bilateral regions of interest were drawn freehand along the border of the entire striatum of serial brain sections from each individual mouse brain to define the representative binding density for the striatum (see Fig. 1a for representations of the coronal sections chosen). Data were linearly fitted to a standard curve, which was then used for calibration with a coefficient (R) greater than 0.99, thereby converting counts per minute per millimeter into nanocurie per milligram tissue. Subsequently, the receptor-bound radioligand densities were calculated using the specific activity of each radioligand as previously described [9]. The experimenter was blinded to all conditions during the analysis.

Fig. 1



Changes in D_1R, D_2R, and D_3R binding in mouse striatum after sleep deprivation. (a) Schematic mouse brain sections showing the rostral to caudal extent of the striatum, the region of interest in which D_1R, D_2R, and D_3R were quantified, across a total of seven sections (modified from [17]). (b) D_1R autoradiography on multiple brain sections through the striatum shown in pseudocolor image intensity. D_1R binding was significantly decreased after sleep deprivation compared with controls (*$P<0.05$, two-tailed *t*-test). (c) D_2R autoradiography on multiple brain sections through the striatum shown in pseudocolor image intensity. D_2R binding did not significantly change after sleep deprivation ($P>0.05$, two-tailed *t*-test). (d) D_3R autoradiography on multiple brain sections through the striatum shown in pseudocolor image intensity. D_3R binding significantly increased after sleep deprivation compared with controls (*$P<0.05$, two-tailed *t*-test). Nonspecific binding was negligible in all three dopamine receptor binding assays. NSB, nonspecific binding; SD, sleep deprivation; str, striatum. Scale bars = 1 mm. Pseudocolor scale = 0 to 100% relative intensity.

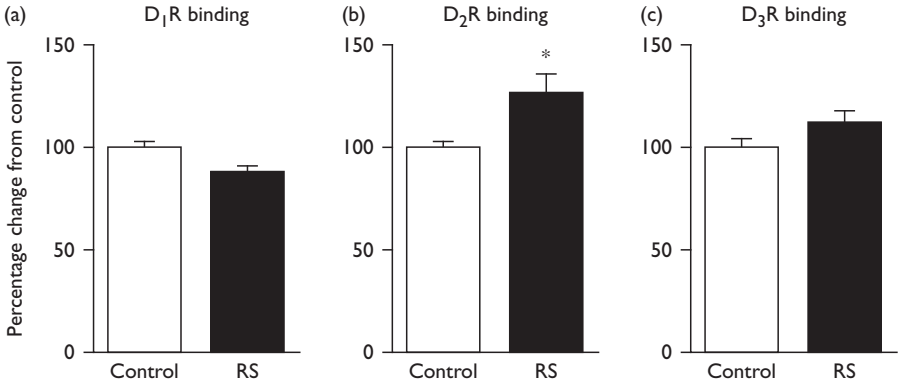
Results

Sleep deprivation

Quantitative receptor autoradiography showed that [^{3}H]SCH 23390, [^{3}H]raclopride, and [^{3}H]WC-10 labeled respective D_1R, D_2R, and D_3R throughout the rostral to caudal regions of the mouse striatum (Fig. 1a–d).

Compared with age-matched controls, sleep-deprived mice showed an approximate 15% significant decrease in D_1R density in the striatum [$87 \pm 3\%$ change relative to controls, per femtomoles/milligram (fmol/mg) tissue, mean value ± standard error of the mean (SEM), $P < 0.05$, *t*-test] (Fig. 1b). There was no significant change in D_2 receptor density in the striatum ($105 \pm 4\%$ change relative to controls, per fmol/mg tissue, mean value ± SEM, $P > 0.05$, *t*-test) (Fig. 1c). In contrast, there was nearly a 20% significant increase in D_3R density in the striatum ($119 \pm 6\%$ change relative to controls, per fmol/mg tissue, mean value ± SEM, $P < 0.05$, *t*-test) (Fig. 1d).

Fig. 2



Changes in D_1R, D_2R, and D_3R binding in mouse striatum after restraint stress. (a) D_1R binding was not significantly changed after restraint stress compared with controls ($P > 0.05$, two-tailed *t*-test). (b) D_2R binding significantly increased after restraint stress compared with controls ($^*P < 0.05$, two-tailed *t*-test). (c) D_3R binding was not significantly changed after restraint stress compared with controls ($P > 0.05$, two-tailed *t*-test). RS, restraint stress.

Restraint stress

Compared with age-matched controls, mice that underwent restraint stress showed no significant difference in D_1R density in the striatum (95 ± 3% change relative to controls, per fmol/mg tissue, mean value ± SEM, $P > 0.05$, *t*-test) (Fig. 2a). In contrast, there was a significant increase of approximately 25% in D_2R density in the striatum (126 ± 9% change relative to controls, per fmol/mg tissue, mean value ± SEM, $P < 0.05$, *t*-test) (Fig. 2b). Lastly, there was no significant difference in D_3R density in the striatum (111 ± 5% change relative to controls, per fmol/mg tissue, mean value ± SEM, $P > 0.05$, *t*-test) (Fig. 2c).

It should be noted that this pattern of dopamine receptor changes markedly differs from that seen after sleep deprivation. This suggests that dopamine receptor changes may be specific to sleep deprivation and not merely a byproduct of stress.

Discussion

Until this point, it has been a challenge to elucidate the specific role of D_3R due to lack of selective radioligands available for receptor autoradiography. Our data are the first to show the full complement of D_1R, D_2R, and D_3R changes that occur together in the same animal after chronic sleep deprivation. This is also the first report of the localization of [^{3}H]WC-10, the selective D_3R radioligand, in mouse striatum.

We show that total sleep deprivation for 72 h decreases D_1R and increases D_3R density in the striatum, but has no significant effect on D_2R binding. Only one previous report has examined dopamine receptor changes after sleep deprivation, and this focused on D_1R and D_2R binding after 96 h of selective REM sleep deprivation in rat [18].

One could argue that sleep deprivation is inherently stressful, and stress has well-established effects on dopamine receptor dynamics. However, mice that were subjected to chronic restraint stress did not show the same pattern of dopamine receptor changes that were seen after sleep deprivation. In contrast, stressed mice showed no such changes in D_1R or D_3R densities, but rather, they showed a significant increase in D_2R binding. This finding in stressed mice is consistent with previously published reports showing that both restraint stress and social stress significantly increase D_2R binding, but do not change in D_1R binding in rats [19]. Chronic stress has been reported to enhance dopamine uptake in the striatum, along with alterations in the DAT activity [20]. Changes in DAT and D_2R density may represent homeostatic scaling mechanisms for the modulation of dopaminergic activity following periods of stress.

Dopamine receptors are a family of seven-transmembrane domain, G-protein coupled receptors. D_1-like dopamine receptors (D_1R and D_5R) are positive regulators of cyclic AMP levels [21]. D_1R are thought to be exclusively postsynaptic in location. In contrast, D_2-like dopamine receptors (D_2R, D_3R, and D_4R) inhibit adenylyl cyclase activity in general. D_2R and D_3R are found both presynaptically and postsynaptically [22]. D_1R, D_2R, and D_3R are extensively coexpressed in the striatum. It has been reported that D_1R and D_3R may heterodimerize in the striatum, despite having opposing second messenger signaling cascades [23]. In this study, we observed that D_1R and D_3R changed in opposite directions after sleep deprivation, whereas D_2Rs remained unchanged. This finding invites renewed efforts in elucidating the functional roles of the different dopamine receptor subtypes in the brain.

Volkow *et al.* [7] recently reported decreased [^{11}C]raclopride binding in humans after a single night of total sleep deprivation. It would be interesting to image these participants again after 72 h to see if receptor changes

persist, as we found for D_1R and D_3R density in mice. Our study results are not inconsistent with their finding of decreased [^{11}C]raclopride binding in humans; [^{11}C]raclopride was not used as a quantitative measure of D_2R density, but rather as an indirect measure of endogenous dopamine release [7]. Total sleep deprivation is thought to increase dopamine release in the striatum, whether as a consequence of, or perhaps even to help promote, wakefulness. Indeed, other studies have found that human sleep deprivation increases functional MRI activation in the nucleus accumbens and is associated with risky decision making, which would be consistent with increased dopamine release [24].

The functions of the dopamine D_3R subtype in the brain are still being elucidated. Recent evidence uncovered a link between D_3R and a mouse model of restless legs syndrome, a sleep disorder with a circadian dysregulation of sensory and motor gating which is treated with dopaminergic agonist medications [25]. Other potential functions for D_3R include motor learning, motivation/reward, cognition, and locomotor response [23]. Given our results, future studies should be performed to better elucidate the specific role of D_3R and sleep.

Conclusion

Taken together, our data show that sleep deprivation differentially affects D_1R, D_2R, and D_3R density in the striatum in a pattern that appears to be specific to sleep deprivation as opposed to generalized stress. In particular, D_3R changes may point to a novel role for D_3R in sleep behavior. These data provide further evidence that brain dopaminergic circuits may undergo remodeling during sleep deprivation.

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