Comparison of Brain Activity between Dopamine D2 Receptor-Knockout and Wild Mice in Response to Dopamine Agonist and Antagonist Assessed by fMRI

Jun-ichi Kuriwaki^a Hisao Nishijo^{b,d} Takashi Kondoh^e Teruko Uwano^c
Kunio Torii^e Motoya Katsuki^f Taketoshi Ono^{a,d}

^aMolecular and Integrative Emotional Neuroscience, ^bSystem Emotional Science, ^cIntegrative Neuroscience, Graduate School of Medicine, Toyama Medical and Pharmaceutical University, Sugitani, Toyama; ^dJST, CREST, Tokyo; ^eInstitute of Life Sciences, Ajinomoto Co., Inc., Kawasaki; ^fNational Institute for Basic Biology, Okazaki National Research Institute, Okazaki, Japan

Key Words

Functional MRI · Caudate-putamen · Hippocampal formation · Amygdala · Bromocriptine · Spiperone · Antipsychotic drugs

Abstract

It has been reported that antipsychotic dopamine-D2-receptor (D2R) antagonists affected other neurotransmitter systems. In the present study, the effects of a D2R agonist, bromocriptine, and a D2R antagonist, spiperone, on brain activity were investigated using wild-type mice (WT) with intact D2Rs, and D2R-knockout mice (D2R-KO) lacking D2Rs by functional magnetic resonance imaging. In the WT, flow-weighted signal intensity significantly increased after administration of bromocriptine in the hippocampal formation. In contrast, signal intensity significantly decreased after administration of spiperone in the somatosensory-motor cortices, thalamus, anterior cingulate cortex, caudate-putamen, nucleus accumbens, hippocampal formation, and amygdala.

In the D2R-KO, however, no significant changes were observed after administration of either bromocriptine or spiperone. The present results indicated that the D2R-KO lacked sensitivity to D2R agonist and antagonist in agreement with its genetic defects, which confirmed that the changes in brain activity in the WT after administration of either drug were mediated through D2Rs. These results suggest that antipsychotic D2R antagonists affect activity of the same brain regions of human patients through D2Rs, as observed in the present study. These changes in brain activity might be related to therapeutic efficacy as well as side effects of antipsychotic drugs on schizophrenic patients.

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Introduction

Dopamine constitutes about 80% of the catecholamine content in the brain. Projections originating from the brain areas that synthesize this neurotransmitter give rise

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Dr. Taketoshi Ono
Molecular and Integrative Emotional Neuroscience, Graduate School of Medicine
Toyama Medical and Pharmaceutical University
Sugitani 2630, Toyama 930-0194 (Japan)
Tel. +81 76 4347220, Fax +81 76 4345013, E-Mail onotake@ms.toyama-mpu.ac.jp

to four main axonal pathways: (1) nigrostriatal; (2) mesolimbic; (3) mesocortical, and (4) tuberoinfundibular pathways [1]. These pathways are involved in regulation of a variety of functions including locomotor activity, some aspects of learning and memory, motivated behaviors and neuroendocrine secretion [1–3]. The dopamine system has been the focus of extensive research for several years, mainly because alteration in dopamine neurotransmission is involved, directly or indirectly, in several brain dysfunctions.

Dopamine-D2-receptors (D2Rs), which are located in the brain regions such as the caudate-putamen, olfactory tubercle and nucleus accumbens [1, 4–7], have important roles for the dopaminergic system to manifest its psychomotor effects. Administration of low doses of D2R agonists causes a reduction of locomotor functions, probably by stimulating presynaptic receptors. This should lead to a reduction of dopaminergic cell firing and of dopamine release [1, 8–10]. In contrast, administration of D2R antagonists decreases motor activity. When high doses of these compounds are administered, the animals become cataleptic, maintaining an abnormal position for a period of time [1, 8, 10]. Previous studies of locomotor behavior using D2R-knockout (D2R-KO) mice clearly revealed motor impairments that were characterized by reduced and uncoordinated movements and also by suppression of rearing [1, 11]. Our previous studies also reported that D2R-KO mice displayed a hypoactivity, a slow and creeping movement, and place learning deficits [12, 25].

Recent functional magnetic resonance imaging (fMRI) techniques provide a non-invasive means of visualizing and measuring brain activity. T_2^* -weighted MRI is a method that is based on the blood oxygenation level-dependent (BOLD) contrast caused by paramagnetic deoxyhemoglobin in blood vessels [13, 14]. Preece et al. [15] reported utility of BOLD contrast fMRI as a means of detecting changes in neuronal activity contingent upon the administration of a psychoactive pharmacological agent. Hagino et al. [16], in their BOLD fMRI study, reported the sites of therapeutic action of antipsychotics by comparing effects of acute administration of bromocriptine (BROMO), a D2R agonist, with those of acute administration of haloperidol, a D2R antagonist, on brain activity of unanesthetized rats. The authors suggested that changes in signal intensity after haloperidol injection might be related to its antipsychotic effects, which were mediated through D2 dopamine system. However, it has been reported that haloperidol stimulated systems other than the D2 dopamine system, such as the cholinergic system [17], and changed affinity of neotensin receptor

[18]. Furthermore, effects of haloperidol were blocked by an α -adrenoceptor antagonist [19]. Therefore, it would be interesting if D2R-KO mice were tested with these dopaminergic agents. However, no fMRI measurements after administration of D2 dopaminergic agents have been reported in D2R-KO mice.

In order to elucidate possible relationships between D2Rs in the brain and therapeutic action and side effects of antipsychotic drugs, we investigated effects of acute administration of D2R agonist and antagonist on brain activity of anesthetized wild and D2R-KO mice by using fMRI.

Materials and Methods

Animals

A total of 18 wild-type male mice (WT) and 14 D2R-KO male mice, weighing 28.0 ± 5.0 and 27.6 ± 3.6 g at the start of the experiments, respectively, were used. We obtained the mice from a collaborative laboratory (National Institute for Basic Biology, Okazaki National Research Institute, Okazaki). The generation of D2R-KO mice has been reported previously [12]. Genotyping of the knockout and wild-type animals used in these experiments was determined by polymerase chain reaction (PCR) analysis of products derived from tail DNA. Each strain of animals was divided into four groups: (1) bromocriptine (BROMO) group (5 WT and 3 D2R-KO); (2) vehicle (20% polyethyleneglycol in 0.9% saline solution) of BROMO group (3 WT and 3 D2R-KO); (3) spiperone (SPP) group (5 WT and 4 D2R-KO), and (4) vehicle (saline) of SPP group (5 WT and 4 D2R-KO). All animals were housed individually with free access to food and water in a room where temperature ($23 \pm 1^\circ\text{C}$), relative humidity ($60 \pm 10\%$) and light (09:00–21:00 h) were automatically controlled. All experimental protocols were approved by the Animal Care and Use Committee in our university (Toyama Medical and Pharmaceutical University), and conformed to the NIH and Society for Neuroscience Policy on the use of laboratory animals.

Drugs

BROMO (2-bromocriptine (α -ergocryptine); Sigma, St. Louis, Mo., USA) and SPP (spiperone hydrochloride; Sigma) were used. BROMO was dissolved in 20% polyethyleneglycol in 0.9% saline [20]. SPP was dissolved in 0.9% saline. All drugs were dissolved freshly just before the MRI experiments.

Magnetic Resonance Imaging

An MRI system (SMIS, Guilford, UK), consisting of a 4.7-T/400 horizontal superconducting magnet equipped with actively-shielded gradient coils (60 mT/m, 26-cm inner diameter), was used. Animals were anesthetized with intraperitoneal injection of urethane (1.0 g/kg) and α -chloralose (65 mg/kg), and were cannulated subcutaneously for drug administration. The head of the animal and a homemade volume coil (60 mm diameter) were fixed in a stereotaxic apparatus specially designed for MRI. The stereotaxic apparatus with the volume coil and animal was subsequently transferred into a magnet bore. Tuning and matching of the coil and shimming of the magnetic field were done for image acquisition.

Fig. 1. T₂-weighted brain images (spin-echo images) of anterior (**A**) and posterior (**B**) planes of a wild-type mouse showing location of 22 brain regions subjected to statistical analysis. MOp = Primary motor cortex; ACv = ventral part of the anterior cingulate cortex; CPa = anterior part of the caudate-putamen; Acb = nucleus accumbens; SSsp = primary somatosensory cortex; HF = hippocampal formation; IAM = interanteromedial nucleus of the thalamus; VPL = ventral posterolateral nucleus of the thalamus; CPp = posterior part of the caudate-putamen; AMC = central nucleus of the amygdala; LHA = lateral hypothalamic area.

All the functional MR images were taken in two coronal brain sections at 0.5 mm anterior (anterior plane) and 1.5 mm posterior (posterior plane) to the bregma. These coordinates were deemed most suitable for obtaining images from the major neural structures receiving dopaminergic afferents (the anterior cingulate cortex, caudate-putamen, nucleus accumbens, and central nucleus of the amygdala). To obtain precise anatomical brain maps, on which the activated areas in the flow-weighted MR images were superimposed, two T₂-weighted spin echo images were obtained with conventional spin echo pulse sequence. The settings were: repetition time (TR) = 2,500 ms, echo time (TE) = 30 ms, slice thickness (THK) = 1.4 mm, field of view (FOV) = 17 × 17 mm, matrix = 64 × 64 pixels, number of average = 4, number of slices = 2, slice gap (center-to-center) = 2.0 mm.

After acquiring anatomical images, functional brain images (flow-weighted images) were taken 20, 15, 10 and 5 min before (control images), and 0, 5, 10, 15, 20, 30, 60, 90, 120 and 150 min after the drug injection, by using a gradient echo pulse sequence on the same slices predetermined by anatomical images. The settings were: TR = 100 ms, TE = 6 ms, flip angle = 45°, THK = 1.4 mm, FOV = 17 × 17 mm, matrix = 64 × 64 pixels, number of average = 8, number of slices = 2, slice gap (center-to-center) = 2.0 mm. We selected flow-weighted MRI for functional imaging because flow-weighted MRI is sensitive to in-flow of blood [21, 22]. The method would provide simpler interpretation on physiological and pharmacological changes in brain functions than would conventional T₂*-weighted MRI, in which signal intensity is dependent on blood oxygenation level and hence is determined by the balance of oxygen supply (blood flow; signal increase) and demand (oxygen consumption; signal decrease) [13, 23].

Regions of Interest (ROIs)

For statistical analyses, adequate regions of interest (ROIs) in blocks of 3 × 3 pixels were selected in 11 brain regions (4 regions in the anterior plane and 7 regions in the posterior plane) based on the stereotaxic atlas of mice [24] (fig. 1). The 4 ROIs selected on the anterior plane (A, 0.5 to bregma) were: a ventral part of the anterior cingulate cortex (ACv) (L, ±0.2; DV, +1.6; coordinates of a center of the ROI in the atlas), primary motor cortex (MOp) (L, ±2.3; DV, +1.4), anterior part of the caudate-putamen (CPa) (L, ±1.4; DV, +3.6), and nucleus of accumbens (Acb) (L, ±1.0; DV, +4.4). The 7 ROIs selected on the posterior plane (A, -1.5 to bregma) were: the primary somatosensory cortex (SSsp) (L, ±2.7; DV, +1.0), hippocampal formation (HF) (L, ±1.2; DV, +1.5), interanteromedial nucleus of the thalamus (IAM) (L, ±0.4; DV, +3.5), ventral posterolateral nucleus of the thalamus (VPL) (L, ±2.1; DV, +3.8), posterior part of the caudate-putamen (CPp) (L, ±3.2; DV, +4.1), lateral hypothalamic area (LHA) (L, ±1.2; DV, +5.1), and central nucleus of the amygdala (AMC) (L, ±3.3; DV, +4.4).

Statistical Analysis

Two-dimensional gaussian filter (full width at the half maximal = 2.0 pixels) was applied on each image. Averaged signal intensity in each ROI (3 × 3 pixels) was then measured (fig. 1). Data from the left and right sides in each brain region (data from a total of 18 pixels) were averaged, and further processed for the subsequent data analyses. In each mouse, control signal intensity (pre-injection baseline control) in each ROI was determined from four pre-injection control images acquired at 20, 15, 10 and 5 min before injection. Data from the ROIs were finally expressed as the percent changes in signal intensity relative to the signal intensity of pre-injection baseline controls. The percent changes in signal intensity in each brain region was

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Fig. 2. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in the MOp (**A**) and ACv (**B**) in the anterior planes of the WT (**a**) and D2R-KO (**b**) after subcutaneous injection of BROMO. No significant changes were observed after injection of BROMO in both the WT and D2R-KO.

Fig. 3. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in the CPa (**A**) and Acb (**B**) in the anterior planes of the WT (**a**) and D2R-KO (**b**) after subcutaneous injection of BROMO. No significant changes were observed after injection of BROMO in both the WT and D2R-KO.

Fig. 4. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in the SSa (**A**), HF (**B**) and IAM (**C**) in the posterior planes of the WT (**a**) and D2R-KO (**b**) after subcutaneous injection of BROMO. Significant increase was observed in the HF at 60 and 120 min in the WT. However, no significant changes were observed in the D2R-KO. * $p < 0.01$, significant difference between vehicle- and BROMO-injected groups.

statistically compared between the D2R drug group and its corresponding vehicle group, and between the two D2R drug groups by Bonferroni corrected t test. Results were considered significant if the p value was < 0.01 . All results were expressed as the mean $\pm$ SEM.

Results

Effects of BROMO

Figures 2 and 3 show percent changes in signal intensity in the anterior brain regions after administration of BROMO (5.0 mg/kg) in the WT (a) and D2R-KO (b). In the anterior planes, there were no significant differences

in percent signal intensity between the control and treated animals of both the WT and D2R-KO. Figures 4–6 show percent changes in signal intensity in the posterior brain regions after administration of BROMO (5.0 mg/kg) in the WT (a) and D2R-KO (b). In the WT, BROMO significantly increased signal intensity in the HF at 60 and 120 min after injection, as compared with the vehicle-injected control group. In the D2R-KO, however, there were no significant differences in percent signal intensity between the control and treated animals.

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Fig. 5. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in the VPL (**A**) and CPp (**B**) in the posterior planes of the WT (**a**) and D2R-KO (**b**) after subcutaneous injection of BROMO. No significant changes were observed after injection of BROMO in both the WT and D2R-KO.

Fig. 6. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in the LHA (**A**) and AMC (**B**) in the posterior planes of the WT (**a**) and D2R-KO (**b**) after subcutaneous injection with BROMO. No significant changes were observed after injection of BROMO in both the WT and D2R-KO.

Fig. 7. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in the MOp (**A**) and ACv (**B**) in the anterior planes of the WT (**a**) and D2R-KO (**b**) after subcutaneous injection of SPP. In the WT, a significant decrease compared with the controls was observed in the MOp at 150 min, and ACv at 120–150 min after injection of SPP. In the D2R-KO, however, no significant changes were observed. * $p < 0.01$ and ** $p < 0.001$, significant difference between the vehicle- and SPP-injected groups.

Fig. 8. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in the CPa (**A**) and Acb (**B**) in the anterior planes of the WT (**a**) and D2R-KO (**b**) after subcutaneous injection of SPP. In the WT, a significant decrease compared with the controls was observed in the CPa and Acb at 120–150 min after injection of SPP. In the D2R-KO, however, no significant changes were observed. * $p < 0.01$ and ** $p < 0.001$, significant difference between the vehicle- and SPP-injected groups.

Fig. 9. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in the SSp (**A**), HF (**B**) and IAM (**C**) in the posterior planes of the WT (**a**) and D2R-KO (**b**) after subcutaneous injection of SPP. In the WT, a significant decrease compared with the controls was observed in the SSp and HF at 150 min after injection of SPP. In the D2R-KO, however, no significant changes were observed. * $p < 0.01$, significant difference between the vehicle- and SPP-injected groups.

Effects of SPP

Figures 7 and 8 show percent changes in signal intensity in the anterior brain regions after administration of SPP (0.05 mg/kg) in the WT (a) and D2R-KO (b). In the WT, SPP significantly decreased percent signal intensity in the MOp at 150 min, and ACv, CPa, and Acb at 120–150 min, as compared with the vehicle-injected control group. The decrease in signal intensity in these brain areas occurred slowly and appeared to persist beyond 150 min, the time of the final measurement. In the D2R-KO, however, there were no significant differences in percent sig-

nal intensity between the control and SPP-treated animals.

Figures 9–11 show percent changes in signal intensity in the posterior brain regions after administration of SPP in the WT (a) and D2R-KO (b). In the WT, SPP significantly decreased percent signal intensity in the SSp at 150 min, HF at 150 min, VPL at 90–150 min, CPp at 90–150 min, and AMC at 120–150 min, as compared with the vehicle-injected control group. Consistent with the anterior planes, the decrease in signal intensity in these brain areas occurred slowly and appeared to persist

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Fig. 10. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in VPL (A) and CPp (B) in the posterior planes of the WT (a) and D2R-KO (b) after subcutaneous injection of SPP. In the WT, a significant decrease compared with the controls was observed in the VPL and CPp at 90–150 min after injection of SPP. In D2R-KO, however, no significant changes were observed. * $p < 0.01$, significant difference between the vehicle- and SPP-injected groups.

Fig. 11. Percent changes in signal intensity (SI) ($\pm$ SEM) on functional MR images in LHA (A) and AMC (B) in the posterior plane of the WT (a) and D2R-KO (b) after subcutaneous injection of SPP. In the WT, a significant decrease compared with the controls was observed in the AMC at 120–150 min after injection of SPP. In the D2R-KO, however, no significant changes were observed. * $p < 0.01$, significant difference between the vehicle- and SPP-injected groups.

Fig. 12. Comparison of changes in signal intensity (SI) ($\pm$ SEM) on functional MR images between the BROMO- and SPP-injected groups of the WT in the MOp (A), ACv (B), CPa (C), Acb (D), SSa (E), and HF (F). In the SPP-injected group, a significant decrease compared with the BROMO-injected group was observed in each area at 60–150 min after injection. * $p < 0.01$ and ** $p < 0.001$, significant difference between the BROMO- and SPP-injected groups.

beyond 150 min. Thus, the changes in signal intensity after SPP administration were larger than those after BROMO administration. In the D2R-KO, however, there were no significant differences in percent signal intensity between the control and treated animals.

Comparison of Effects between BROMO and SPP

Figures 12 and 13 show direct comparison of percent changes in signal intensity between the BROMO- and SPP-injected groups of the WT in each area. In the SPP-injected group of the WT, percent changes in signal inten-

sity in all areas significantly decreased at 60–150 min after injection, as compared with the BROMO-injected group of the WT.

Discussion

Effects of the Drugs in D2R-KO

The present results indicated that injection of BROMO increased signal intensity in the HF of the WT. In contrast, injection of SPP decreased signal intensity in the

Fig. 13. Comparison of changes in signal intensity (SI) ($\pm$ SEM) on functional MR images between the BROMO- and SPP-injected groups of the WT in the IAM (**A**), VPL (**B**), CPp (**C**), LHA (**D**), and AMC (**E**). In the SPP-injected group, a significant decrease compared with the BROMO-injected group was observed in each area at 60–150 min after injection. * $p < 0.01$ and ** $p < 0.001$, significant difference between the BROMO- and SPP-injected groups.

many brain regions (MOp, SSp, VPL, ACv, CPa, CPp, Acb, HF and AMC) of the WT. However, in the D2R-KO, percent signal intensity did not change after administration of BROMO nor SPP.

In the present study, we used D2R-deficient mice generated by a gene-targeting method. The D2R-KO had few D2Rs in comparison with the WT [12]. Before the experiments, we compared body weight and brain size of the D2R-KO with those of the WT. The mean body weight of the WT was slightly larger than that of the D2R-KO, but the difference was not significant (Student's *t* test, $p >$

0.05). The brain sizes measured by MRI were not significantly different between the WT and D2R-KO (data not shown). Furthermore, light microscopic investigation indicated that an apparent cell loss was not observed in the brains of the D2R-KO (data not shown). These results suggest that the differences in brain activity between the WT and D2R-KO were attributed to functional differences (i.e., differences in neuronal activity), but not to structural changes. Consistent with this idea, a recent neurophysiological study reported that neuronal activity in the nucleus accumbens of the D2R-KO was different

from that of the WT in a situation when dopaminergic activity increased [25]. Taken together, these results strongly suggest that the changes in signal intensity after administration of D2R agonist and antagonist in the WT were due to changes in neuronal activity mediated through D2Rs.

Acute BROMO Injection

The percent changes in signal intensity significantly increased in the HF at 60 and 120 min after injection of BROMO in the WT. This suggests that D2R agonist increased brain activity and local cerebral glucose utilization (LCGU) in this region. However, no changes in signal intensity were observed in the caudate-putamen in the present study. Consistent with the present study, Pizzolato et al. [9] reported no LCGU changes in the caudate-putamen after administration of BROMO (20 mg/kg) although BROMO slightly decreased LCGU 2 h after administration in some brain regions, but increased from 3 to 4 h in many brain regions. Our previous study in rats also confirmed the present results, i.e., no changes in signal intensity in the caudate-putamen [16]. Furthermore, the present results indicated that acute BROMO administration increased percent changes in signal intensity in the HF with less intimate connections to the dopaminergic system (see below for discussion).

There might be some possible reasons why BROMO did not change signal intensity as much as SPP. First, urethane, used as anesthetics in the present study, was reported to decrease excitability of the striatal neurons [26] although it did not change extracellular DA levels in the striatum [27]. This might decrease excitatory effects of D2-agonist (BROMO) on some striatal neurons [28, 29]. Second, it has been reported that D2 receptors might synergistically interact with D1 receptors to excite striatal neurons [30, 31]. Therefore, administration of D2 agonist alone might be not sufficient to excite some striatal neurons. Further studies are necessary to determine whether these possibilities are correct.

Acute SPP Injection

The percent changes in signal intensity significantly decreased in the MOp, SSsp, ACv, VPL, CPa, CPp, Acb, HF, and AMC after injection of SPP in the WT. This suggests that D2R antagonist decreased brain activity and LCGU in these regions. Our previous study using awake rats also reported a decrease in percent changes in signal intensity after injection of haloperidol, a D2R antagonist [16]. These findings are consistent with McCulloch et al. [4], in which LCGU decreased in the cortex, caudate-

putamen, HF and other brain regions after acute administration of haloperidol, except the Acb in which LCGU increased. We measured signal intensity in the core of the Acb, which resembles neuroanatomically and neurochemically the caudate-putamen [32] and is functionally different from the shell of the Acb [33–36]. The discrepancy between the present and previous studies might be ascribed to this regional difference, as well as differences in the animal species and drugs used in the studies.

Effects of Dopamine D2 Receptor Agonist and Antagonist on the HF

Density of D2-like receptors is relatively low in the HF although the HF receives dopaminergic afferents from the mesolimbic dopaminergic system in the midbrain. However, both the D2R agonist and antagonist induced changes in signal intensity in the HF. These receptor agonist and antagonist might not only directly but also indirectly affect the HF; these receptor agents might directly act on D2Rs in the neocortical or other limbic neurons that project to the HF, which in turn changed activity in the HF. Furthermore, both D1 and D2 dopamine receptors in the stratum lacunosum-moleculare of the HF CA1 subregion, where the perforant pathway terminates, modulated glutamatergic inputs from the perforant pathway [37].

Yang and Mogenson [38] reported that signal transmission from the HF might be regulated by a D2R mechanism in the nucleus accumbens, presumably mediated by the converging mesolimbic dopaminergic inputs from the ventral tegmental area. Wilkerson and Levin [39] reported that the dying back of dopamine pathways results in decreased levels of dopamine in the HF, which may account for some of the cognitive dysfunctions in Parkinson's disease. Furthermore, there is a substantial loss of D2Rs in the HF, which may also be related to cognitive dysfunction in Alzheimer's disease [39]. These results suggest that HF cognitive functions are closely related to D2Rs. Consistent with these previous results, the present results indicated that the D2R dopaminergic system efficiently modulated neural activity in the HF. Furthermore, dysfunctions of this dopamine transmission in the HF might be associated with schizophrenia in which various cognitive functions were disturbed [37].

The present results suggest that the HF, ACv, caudate-putamen, Acb, AMC and thalamus are the most efficient sites of action of antipsychotics, where pathophysiology of schizophrenia has been implicated [40–42]. Furthermore, the present results could indicate activity changes in the three important areas responsible for therapeutic effects

and side action of the antipsychotics [43]; the prefrontal cortex (ACv) receiving afferents from the mesocortical dopamine pathway, limbic system (Acb, AMC, HF) receiving afferents from the mesolimbic dopamine pathway, and caudate-putamen (CPa, CPp) receiving afferents from the nigrostriatal dopamine pathway. The present methods might provide an attractive opportunity for new research on drug treatment of schizophrenia.

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References

- Vallone D, Picetti R, Borrelli E: Structure and function of dopamine receptors. *Neurosci Biobehav Rev* 2000;24:125–132.
- Jaber M, Robinson SW, Missale C, Caron MG: Dopamine receptors and brain function. *Neuropharmacology* 1996;35:1503–1519.
- Missale C, Nash SR, Robinson SW, Jaber M, Caron MG: Dopamine receptors: From structure to function. *Physiol Rev* 1998;78:189–225.
- McCulloch J, Savaki HE, Sokoloff L: Distribution of effects of haloperidol on energy metabolism in the rat brain. *Brain Res* 1982;243:81–90.
- Pizzolato G, Soncrant TT, Larson DM, Rapoport SI: Reduced metabolic response of the rat brain to haloperidol after chronic treatment. *Brain Res* 1985;337:1–9.
- Pizzolato G, Soncrant TT, Rapoport SI: Haloperidol and cerebral metabolism in conscious rat: Relation to pharmacokinetics. *J Neurochem* 1984;43:724–732.
- Wamsley JK, Gehlert DR, Filloux FM, Dawson TM: Comparison of the distribution of D1 and D2 dopamine receptors in the rat brain. *J Chem Neuroanat* 1989;2:119–137.
- Jackson DM, Westlind-Danielsson A: Dopamine receptors: Molecular biology, biochemistry and behavioural aspects. *Pharmacol Ther* 1994;64:291–369.
- Pizzolato G, Soncrant TT, Rapoport SI: Time-course and regional distribution of the metabolic effects of bromocriptine in the rat brain. *Brain Res* 1985;341:303–312.
- Seeman P, Van Tol HHM: Dopamine receptor pharmacology. *Trends Pharmacol Sci* 1994;15:264–270.
- Kelly MA, Rubinstein M, Phillips TJ, Lessov CN, Burkhart-Kasch S, Zhang G, Bunzow JR, Fang Y, Gerhardt GA, Grandy DK, Low MJ: Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. *J Neurosci* 1998;18:3470–3479.
- Yamaguchi H, Aiba A, Nakamura K, Nakao K, Sakagami H, Goto K, Kondo H, Katsuki M: Dopamine D2 receptor plays a critical role in cell proliferation and proopiomelanocortin expression in the pituitary. *Genes Cells* 1996;1:253–268.
- Ogawa S, Lee TM, Kay AR, Tank DW: Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci USA* 1990;87:9868–9872.
- Ogawa S, Menon RS, Tank DW, Kim SG, Merkle H, Ellermann JM, Ugurbil K: Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. *Biophys J* 1993;64:803–812.
- Preece M, Mukherjee B, Huang CLH, Hall LD, Leslie RA, James MF: Detection of pharmacologically mediated changes in cerebral activity by functional magnetic resonance imaging: The effects of sulpiride in the brain of the anaesthetized rat. *Brain Res* 2001;916:107–114.
- Hagino H, Tabuchi E, Kurachi M, Saitoh O, Sun Y, Kondoh T, Ono T, Torii K: Effects of D2 dopamine receptor agonist and antagonist on brain activity in the rat assessed by functional magnetic resonance imaging. *Brain Res* 1988;813:367–373.
- Li R, Wing LL, Kirch DG, Wyatt RJ, Chuang DM: Effects of chronic nicotine and haloperidol administration on muscarinic receptor-mediated phosphoinositide turnover in rat brain slices. *Psychopharmacology* 1992;109:248–250.
- Holtom PE, Needham PL, Bennett GW, Aspley S: Chronic, but not acute, dosing of antipsychotic drugs alters neurotensin binding in rat brain regions. *Br J Pharmacol* 2000;131:990–996.
- Borda T, Genaro AM, Cremaschi G: Haloperidol effect on intracellular signals system coupled to α_1 -adrenergic receptor in rat cerebral frontal cortex. *Cell Signal* 1999;11:293–300.
- Van den Buuse M, Lambrechts AC: Bromocriptine-induced decrease in blood pressure in conscious spontaneously hypertensive rats: Evidence for a peripheral site of action. *J Pharm Pharmacol* 1989;41:644–646.
- Bradley WG: Flow phenomena; in *Magnetic Resonance Imaging*. St Louis, Mosby, 1988, pp 359–374.
- Detre J, Leigh JS, Williams DS, Koretsky P: Perfusion imaging. *Magn Res Med* 1992;23:37–45.
- Ogawa S, Tank DW, Menon R, Ellermann JM, Kim SG, Merkle H, Ugurbil K: Intrinsic signal changes accompanying sensory stimulation: Functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci USA* 1992;89:5951–5955.
- Hof PR, Young WG, Bloom FE, Belichenko PV, Celio MR: *Comparative Cytoarchitectonic Atlas of the C57BL/6 and 129/Sv Mouse Brains*. Amsterdam, Elsevier, 2000.
- Tran AH, Tamura R, Uwano T, Kobayashi T, Katsuki M, Matsumoto G, Ono T: Altered accumbens neural response to prediction of reward associated with place in dopamine D2 receptor knockout mice. *Proc Natl Acad Sci USA* 2002;99:8986–8991.
- West MO: Anesthetics eliminate somatosensory-evoked discharges of neurons in the somatotopically organized sensorimotor striatum of the rat. *J Neurosci* 1998;18:9055–9068.
- Chapman CD, Gazzara RA, Howard SG: Effects of phencyclidine on extracellular levels of dopamine, dihydroxyphenylacetic acid and homovanillic acid in conscious and anesthetized rats. *Neuropharmacology* 1990;29:319–325.
- Hu XT, Wachtel SR, Galloway MP, White FJ: Lesions of the nigrostriatal dopamine projection increase the inhibitory effects of D1 and D2 dopamine agonists on caudate-putamen neurons and relative D2 receptors from the necessity of D1 receptor stimulation. *J Neurosci* 1990;10:2318–2329.
- Piercey MF, Hyslop DK, Hoffman WE: Excitation of type II caudate neurons by systemic administration of dopamine agonists. *Brain Res* 1996;706:249–258.
- Shi WX, Smith PL, Pun CLP, Millet B, Bunney BS: D1–D2 interaction in feedback control of midbrain dopamine neurons. *J Neurosci* 1997;17:7988–7994.
- Warenycia MW, McKenzie GM: Excitation of striatal neurons by dexamphetamine is not abolished by either chloral hydrate or urethane anaesthesia. *Neuropharmacology* 1988;27:1309–1312.
- Groenewegen HJ, Wright CI, Beijer AV, Voorn P: Convergence and segregation of ventral striatal inputs and outputs. *Ann NY Acad Sci* 1999;877:49–63.

- 33 Chiara GD: Nucleus accumbens shell and core dopamine: Differential role in behavior and addiction. *Behav Brain Res* 2002;137:75–114.
- 34 Heimer L, Alheid GF, de Olmos JS, Groenewegen HJ, Haber SN, Harlan RE, Zahm DS: The accumbens: Beyond the core-shell dichotomy. *J Neuropsychiat Clin Neurosci* 1997;9:354–381.
- 35 Marinelli M, Piazza PV: Interaction between glucocorticoid hormones, stress and psychostimulant drugs. *Eur J Neurosci* 2002;16:387–394.
- 36 Rebec GV: Real-time assessments of dopamine function during behavior: Single-unit recording, iontophoresis, and fast-scan cyclic voltammetry in awake, unrestrained rats. *Alcohol Clin Exp Res* 1998;22:32–40.
- 37 Otmakhova NA, Lisman JE: Dopamine selectively inhibits the direct cortical pathway to the CA1 hippocampal region. *J Neurosci* 1999;19:1437–1445.
- 38 Yang CR, Mogenson GJ: Hippocampal signal transmission to the pedunculopontine nucleus and its regulation by dopamine D2 receptors in the nucleus accumbens: An electrophysiological and behavioural study. *Neuroscience* 1987;23:1041–1055.
- 39 Wilkerson A, Levin ED: Ventral hippocampal dopamine D1 and D2 systems and spatial working memory in rats. *Neuroscience* 1999;89:743–749.
- 40 Carlsson A: The current status of the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* 1988;1:179–186.
- 41 Carlsson M, Carlsson A: Interactions between glutamatergic and monoaminergic systems within the basal ganglia – Implications for schizophrenia and Parkinson's disease. *Trends Neurosci* 1990;13:272–276.
- 42 Shenton ME, Dickey CC, Frumin M, McCarley RW: A review of MRI findings in schizophrenia. *Schizophr Res* 2001;49:1–52.
- 43 Stahl SM: *Essential Psychopharmacology: Neuroscientific Basis and Practical Applications*, ed 2. Cambridge, Cambridge University Press, 2000.