whereby too much or too little stimulation of prefrontal D1 receptors leads to working memory deficits. D1 receptor stimulation had a suppressive effect on the PFC neural activities involved in a spatial working memory task. Moderate D1 receptor stimulation spatially tunes PFC neurons that process target signals by preferentially suppressing nontarget (noisy) neural activities, whereas excessive D1 receptor stimulation induces nonselective suppression of PFC neural activities irrespective of whether the neural activities are task related or not (Vijayraghavan *et al*, 2007).

Animal studies have suggested that the inverted U-shaped principle of D1 receptor stimulation mediating working memory does not necessarily apply to other prefrontal functions (Floresco and Magyar, 2006). In fact, except for WCST, we did not find any association between D1 receptor availability and prefrontal functions less dependent on the working memory process (word fluency task by phonetic or semantic cues and problem-solving test; Takahashi *et al*, 2008).

Recently, McNab *et al* (2009) showed the quadratic relation between the improvement of working memory capacity by training and the change in D1 receptor availability induced by training, although greater reduction in D1 receptor availability was associated with greater improvements in working memory capacity within the measured range. However, a recent study showed that age-related reduction in D1 receptor availability in PFC was associated with age-related reduction in working memory performance and PFC activation during working memory load (Bäckman *et al*, 2011), indicating that other factors besides D1 receptor availability, such as cerebrovascular pathology, could influence the PFC functions and PFC activation during working memory load in older adults. Furthermore, although [^{11}C]SCH23390 and [^{11}C]NNC112 are selective radioligands for D1 receptors, they have some affinity for 5HT2A receptors. 5HT2A receptor density in the striatum is negligible compared with D1 receptor density, whereas 5HT2A receptor density is not negligible in the extrastriatal regions. Previous reports in the literature have indicated that their affinity for 5HT2A receptors relative to D1 receptors is negligible, and recent *in-vivo* studies reported that ~10% to 25% of the cortical signals of these radioligands were due to binding to 5HT2A receptors. Thus, cautious interpretation of the extrastriatal findings regarding these radioligands is recommended (Ekelund *et al*, 2007; Slifstein *et al*, 2007).

In line with our previous study (Takahashi *et al*, 2007), we also found that D2 receptor availability in the hippocampus (HPC) was positively correlated not only with episodic memory ability but also with WCST performance (Takahashi *et al*, 2008). Patients with lesions in HPC sometimes show deficits in WCST (Corkin, 2001; Igarashi *et al*, 2002). These observations suggest that hippocampal D2 receptors could modulate PFC activity by the HPC–PFC pathway, which has a significant role in the cognitive process (Laroche *et al*, 2000; Thierry *et al*, 2000). Accumulating evidence has suggested the modulatory effects of dopamine on HPC–PFC interactions (Aalto *et al*, 2005; Goto and Grace, 2008; Seamans *et al*, 1998; Tseng *et al*, 2007). Conceivably, dopamine influences PFC neurons directly by prefrontal D1 receptors and indirectly by hippocampal D2 receptors via the HPC–PFC pathway. Supporting the importance of hippocampal D2 receptors in PFC functions, MacDonald *et al* (2009) reported that lower D2 receptor availability in HPC was associated with greater intraindividual variability in episodic memory and executive function, indicating that lower D2 receptor-mediated transmission in HPC leads to noisy neural information processing and results in unstable episodic memory and executive functions.

Müller *et al* (1998) reported that the systemic administration of the mixed D1/D2 agonist pergolide facilitated working memory while the selective D2 agonist bromocriptine had no effect. However, there is converging evidence from human and animal studies to suggest the involvement of D2 receptors in cognitive functions. It was reported that the systemic administration of the D2 agonist bromocriptine in human improved cognitive functions including working memory and executive functions (McDowell *et al*, 1998), and the administration of the D2 antagonist sulpiride impaired those functions (Mehta *et al*, 1999). In an animal study, mice lacking D2 receptors were reported to have a working memory deficit (Glickstein *et al*, 2002). These studies, however, did not reveal the regions most responsible for these effects. Moreover, although the involvement of D1 receptors in working memory is widely recognized, it was not clear whether D1 receptor stimulation alone or the combination of D1 and D2 receptor stimulation is most effective. Positron emission tomography findings including ours suggested that orchestration of prefrontal D1 receptors and hippocampal D2 receptors might be necessary for normal prefrontal functions (MacDonald *et al*, 2009; Takahashi *et al*, 2007, 2008).

Positron emission tomography imaging of D1 and D2 receptors and amygdala function

The amygdala has a central role in processing affective stimuli, and in particular, threatening stimuli in the brain (LeDoux, 2000). The amygdala receives a moderate innervation of dopaminergic fibers (Asan, 1998), and dopamine D1 and D2 receptors are moderately expressed in this region (Ito *et al*, 2008). Dopamine release in the amygdala is increased in response to stress (Inglis and Moghaddam, 1999). It has been shown in animal studies that dopamine potentiates the response of the amygdala

by augmenting excitatory sensory input and attenuating inhibitory prefrontal input to the amygdala (Rosenkranz and Grace, 2002). A human functional magnetic resonance imaging (fMRI) study reported that dopaminergic drug therapy such as levodopa or dopamine agonists partially restored amygdala activation due to emotional task in Parkinson's disease patients who showed no significant amygdala activation during drug-off states (Tessitore *et al*, 2002). In addition, another fMRI study of healthy volunteers has demonstrated that amphetamine potentiated the response of the amygdala during an emotional task (Hariri *et al*, 2002). More recently, Kienast *et al* (2008) reported that dopamine storage capacity in human amygdala, measured with 6-[(^{18})F]fluoro-L-DOPA PET, was positively correlated with fMRI signal changes in the amygdala. However, contribution of dopamine D1 and D2 receptors to amygdala activation in response to affective stimuli is unknown in human. To investigate the relation between amygdala activation and dopamine receptor subtype, we conducted a multimodal *in-vivo* neuroimaging study in which dopamine D1 and D2 receptor availabilities in the amygdala were measured with PET, and amygdala activation in response to fearful stimuli was assessed by fMRI (Takahashi *et al*, 2010*b*). Healthy male subjects, a different cohort from that of our study described in the previous section, underwent fMRI for measuring the amygdala response to fearful faces, after which both D1 and D2 receptors in the amygdala were measured using PET with [^{11}C]SCH23390 and [^{11}C]FLB457, respectively.

Although robust bilateral amygdala activations induced by fearful faces were identified in a group analysis, there was considerable individual difference in the degree of amygdala activation. Similarly, although moderate levels of D1 and D2 receptors in the amygdala were measured, notably high variances in both receptor availabilities were observed. Importantly, D1 receptor availability in the amygdala was not correlated with D2 receptor availability in the amygdala. Both voxelwise statistical parametric mapping analysis and regions of interest analysis revealed that blood oxygen level-dependent signals in the amygdala induced by fearful faces were positively correlated with D1 receptor availability, but not with D2 receptor availability, in the amygdala (Figures 2A and 2B; Takahashi *et al*, 2010*b*). That is, individuals with high D1 receptor density in the amygdala tend to show greater amygdala activation in response to fearful stimuli.

In rat studies, Rosenkranz and Grace (2002) showed that dopamine enhances the response of the amygdala by augmenting excitatory sensory input via dopamine D2 receptor stimulation and attenuating inhibitory prefrontal input to the amygdala through dopamine D1 receptor stimulation. More recently, several studies showed that both D1 and D2 receptor stimulations directly enhanced the excitability of amygdala projection neurons via postsynaptic mechanism (Kroner *et al*, 2005;

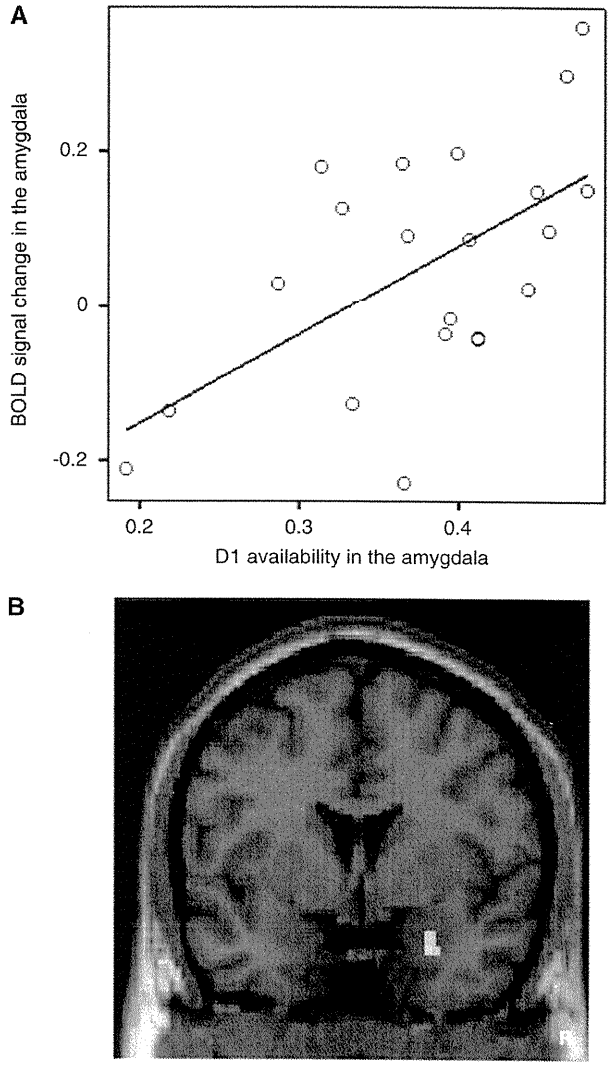

Figure 2 (**A**) Regions of interest (ROIs) correlation analysis revealed significant positive correlations between D1 receptor availability in the amygdala and the degree of amygdala activation. (**B**) Statistical parametric mapping (SPM) correlation analysis also revealed similar correlations. R indicates right.

Rosenkranz and Grace, 2002; Yamamoto *et al*, 2007). Amygdala projection neurons are under inhibitory control by GABAergic interneurons (Royer *et al*, 1999). Both projection neurons and interneurons in the amygdala express dopamine D1 and D2 receptors (Rosenkranz and Grace, 1999). Dopamine and D1 receptor agonist have been shown to augment interneuron excitability and increase the frequency of inhibitory postsynaptic current in amygdala projection neurons (Kroner *et al*, 2005). This is a counterintuitive result, considering the fact that dopamine disinhibits amygdala response *in vivo*. However, Marowsky *et al* (2005) found that a

subpopulation of amygdala interneurons (paracapsular intercalated cells), located between the major input and output stations of the amygdala, is suppressed by dopamine through D1 receptor stimulation. Dopamine D2 receptors also have a role in disinhibiting amydala response by decreasing inhibition onto projection neurons and increasing inhibition onto interneurons (Bissiere *et al*, 2003). As described above, not only dopamine D1 but also D2 receptors contribute to potentiating amygdala response via various mechanisms. In fact, our previous pharmacological fMRI study reported that systemic administration of selective dopamine D2 receptor antagonist attenuated amygdala activation in response to fearful stimuli (Takahashi *et al*, 2005). However, as selective dopamine D1 receptor antagonist for clinical use is not available, we cannot directly compare which D1 or D2 antagonist is more efficient in attenuating amygdala response.

Using a multimodality *in-vivo* neuroimaging approach and dual radioligands, we could for the first time directly compare amygdala dopamine D1 and D2 receptor availabilities with amygdala response evoked by fearful stimuli in human. Although the more detailed mechanism needs to be clarified in future investigations including animal studies, our study suggested that dopamine D1 receptors have a major role in the overall potentiation of amygdala response. At the behavioral level, a number of animal studies have reported that systemic and local applications of D1 agonist (or antagonist) into the amygdala potentiate (or decrease) fear response in animals. Although some studies reported that applications of D2 agonist and antagonist induced similar effects, the results were less consistent compared with D1-mediated effects (for review, see de la Mora *et al*, 2010 and Pezze and Feldon, 2004). Thus, our finding could be regarded as being consistent with previous behavioral pharmacological studies. The combination of PET molecular imaging and fMRI seems to represent a powerful approach for understanding molecular functions in affective neuroscience.

Positron emission tomography imaging of D1 and D2 receptors and decision making under risk

Decision making under risk has been studied in philosophy, psychology, and economics throughout the last century. Normative economic theories (e.g., expected utility theory) assume that individuals are rational decision makers and have purely self-regarding preferences. However, we sometimes make boundedly rational decisions (altruistic behavior, moral judgment, gamble, etc.), which are not accounted for by normative economic theories. Behavioral or experimental economics studies have shown a substantial body of field and empirical evidence that decision makers systematically depart from Camerer and Loewenstein (2004). One type of systematic departure is that subjective weights on probabilities appear to be nonlinear: people often overestimate low probabilities (e.g., playing lotteries) and underestimate high probabilities. A leading alternative to the expected utility theory is the prospect theory (Tversky and Kahneman, 1992). The central feature of the prospect theory is nonlinear probability weighting. Objective probabilities, p, are transformed nonlinearly into decision weights $w(p)$ by a weighting function. In an inverse S-shaped nonlinear weighting function, low probabilities are overweighted and moderate-to-high probabilities are underweighted. The function neatly explains the typically observed pattern of risk seeking for low-probability gain and risk aversion toward high-probability gain.

A synthesis of economics and neuroscience is called neuroeconomics. Neuroeconomics fMRI studies have demonstrated the neural basis for boundedly rational decision makings under risk, including some features of the prospect theory (De Martino *et al*, 2006; Tom *et al*, 2007). A deeper question is how modulatory neurotransmission is involved in the central process of these boundedly rational decision makings (Fox and Poldrack, 2009; Rangel *et al*, 2008; Trepel *et al*, 2005). Investigation of the relationship between the dopamine system and prospect theory seems promising, considering the fact that dopamine is linked to risk-seeking behavior (Leyton *et al*, 2002) and is involved in disrupted decision making observed in neuropsychiatric disorders such as drug/gambling addiction and Parkinson's disease (Steeves *et al*, 2009; Zack and Poulos, 2004). Based on the circumstantial findings, Trepel *et al* (2005) speculated in a thoughtful review that dopamine transmission in the striatum might be involved in shaping probability weighting. To test this speculation, 18 healthy male subjects were studied for D1 receptors with [^{11}C]SCH23390 PET, and 18 other healthy male subjects were studied for striatal D2 receptors with [^{11}C]raclopride PET (Takahashi *et al*, 2010*a*). To estimate decision weight, certainty equivalents were determined outside the PET scanner, based on the staircase procedure suggested by Tversky and Kahneman (1992). A gamble's certainty equivalent is the amount of sure payoff at which a player is indifferent between the sure payoff and the gamble. Participants were presented with options between a gamble and a sure payoff on a computer monitor. Gambles were presented that had an objective probability P of paying a known outcome x (and paying zero otherwise). Multiple gambles with different combinations of P and x were used. In each trial, the participants chose between a gamble and a sure payoff according to their preferences. Each time a choice was made between a gamble and a sure payoff in a trial, the amount of a sure payoff in the next trial was adjusted and eight trials per each gamble were

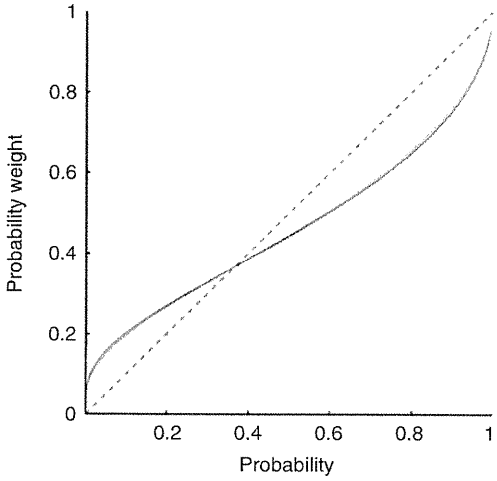

Figure 3 Average fitted probability-weighting function. Red line represents the first group with D1 receptors investigated, and black line the second group with striatal D2 receptors investigated.

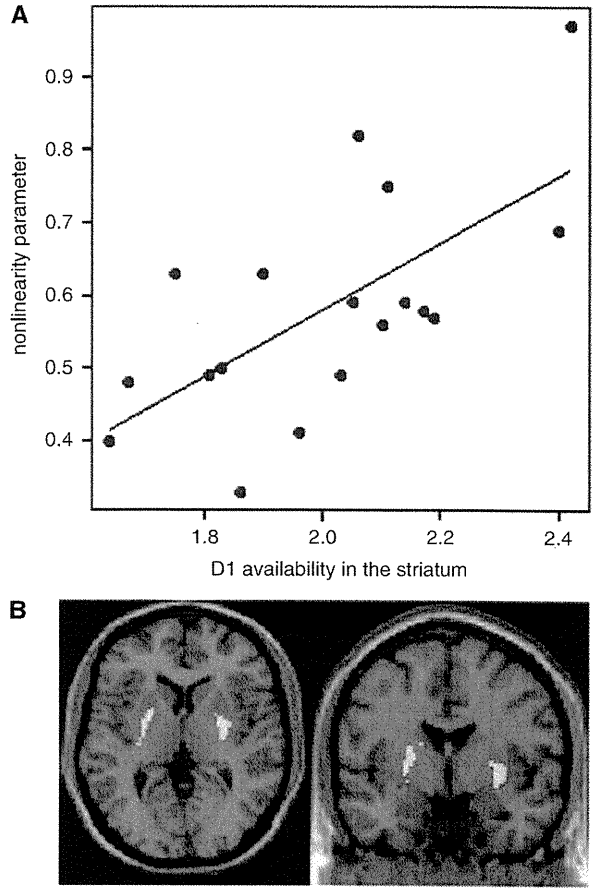

Figure 4 Correlation between nonlinearity of probabilities weighting and D1 receptor availability in the striatum. (**A**) Plots and regression line of correlation between α (nonlinearity parameter) and D1 receptor availability in the putamen ($r = 0.66$, $P = 0.003$). (**B**) Image showing regions of correlation between nonlinearity parameter of weighting function and D1 receptor availability in the striatum.

iterated to successively narrow the range including the certainty equivalents. On the basis of this certainty equivalents estimation experiment, we estimated probability weighting using the one-parameter function derived axiomatically by Prelec (1998), $w(p) = \exp\{-(\ln(1/p))^{\alpha}\}$ with $0 < \alpha < 1$. This $w(p)$ function has an inverted S-shape with a fixed inflection point at $p = 1/e = 0.37$ (at that point the probability $1/e$ also receives decision weight $1/e$). Nonlinearity is fully captured by a single parameter α. A smaller value of α (closer to 0) means a more nonlinear inflected weighting function and a higher value (closer to 1) means a more linear weighting function. At $\alpha = 1$, the function is linear.

In the first group, with D1 receptors investigated, mean (s.d.) α of the weighting function was 0.58 (0.16). In the second group, with striatal D2 receptors investigated, mean (s.d.) α was 0.56 (0.19), indicating that the two groups were comparable. Averaged weighting functions of the two groups are shown in Figure 3 (Takahashi *et al*, 2010*a*). Both regions of interest and voxel-by-voxel statistical parametric mapping analyses revealed significant positive correlation between striatal D1 receptor availability and the nonlinearity parameter α of weighting function (Figures 4A and 4B; Takahashi *et al*, 2010*a*). That is, people with lower striatal D1 receptor availability tend to show more pronounced overestimation of low probabilities and underestimation of high probabilities. It has been suggested that emotional responses to gambles influence weighting. In particular, the overweighting of low-probability gains may reflect hope of winning and the underweighting of high-probability gains may reflect fear of losing a 'near sure thing' (Trepel *et al*, 2005). One study supportive of this hypothesis found more nonlinear weighting functions for gambles over emotional outcomes (kisses and shocks) than over money (Rottenstreich and Hsee, 2001). In this sense, individuals with lower striatal D1 receptor availability might be interpreted as showing more 'emotional' decision making.

A neuroeconomics fMRI, using a simpler exposure-choice paradigm, showed that Prelec's nonlinearity parameter α was negatively correlated with striatal activity during reward anticipation under risk (Hsu *et al*, 2009). That is, people with a greater degree of nonlinearity in striatal activation to anticipated reward tend to overestimate low probabilities (to be risk seeking) and underestimate high probabilities (to be risk averse). Although the mechanism(s) linking the fMRI finding to our PET finding needs to be clarified in future investigations, our molecular imaging approach allows us to broaden our understanding of the neurobiological mechanism underlying decision making under risk beyond the knowledge attained by neuroeconomics fMRI.

Functional significance of individual difference in D1 receptors

All of our three studies mentioned above showed that individual differences in D1 receptor availability in the brain predicted the individual differences in brain functions (working memory/set shifting, emotional reaction, and decision making under risk) better than that of D2 receptor availability (Takahashi *et al*, 2008, 2010*a*,*b*). We do not think that dopamine D2 receptors have minimal roles in these brain functions. However, can we learn something from these studies showing the predominance of D1 receptors in terms of predicting these brain functions?

Dopamine neurons are known to show tonic firing and phasic (burst) firing, and in turn tonic and phasic dopamine release are induced, respectively (Grace, 1991; Grace *et al*, 2007). Phasic dopamine release in the striatum occurs during reward and reward-predicting stimuli (Grace, 1991; Schultz, 2007*b*). Phasic dopamine release in the amygdala is also induced in response to stress or emotional stimuli (Inglis and Moghaddam, 1999). Although both tonic and phasic dopamine release are necessary for PFC functions, phasic dopamine release has a crucial role in working memory and set shifting (Braver *et al*, 1999; Phillips *et al*, 2004). Thus, phasic dopamine release seems to be important for the brain functions that we investigated (working memory/set shifting, emotional reaction, and decision making under risk).

It has been shown that D1 receptors have much less affinity to endogenous dopamine than D2 receptors (Richfield *et al*, 1989). Furthermore, cortical and striatal D1 receptors are known to be predominantly extrasynaptic (Caille *et al*, 1996; Smiley *et al*, 1994). These facts suggest that D1-mediated neurotransmission is mainly governed by volume transmission (Dreher and Burnod, 2002; Garris *et al*, 1994), which might be induced by the phasic dopamine release from axonal terminals (Schultz, 2007*a*). Therefore, it can be suggested that available D1 receptors are preferentially stimulated by phasically released DA, whereas low-level baseline tonic dopamine release is sufficient for stimulating D2 receptors (Frank *et al*, 2007; Schultz, 2007*b*). A recent computational model also showed that phasic dopamine release primarily increases D1 occupancy, whereas D2 occupancy was less affected (Dreyer *et al*, 2010). Thus, these considerations lead us to believe that the variability of available D1 receptors might be more associated with individual differences in brain functions that require phasic dopamine release.

Clinical implications

Our previous PET study using [^{11}C]SCH23390 revealed that, compared with normal controls, D1 receptors in PFC were decreased in schizophrenia, which was associated with poor performance on WCST (Okubo *et al*, 1997*b*). However, another PET study using [^{11}C]NNC112 reported that increased D1 receptors in PFC were associated with working memory deficits in schizophrenia (Abi-Dargham *et al*, 2002). The same research group recently replicated increased D1 receptors in PFC of drug-naive schizophrenia patients (Abi-Dargham *et al*, 2011). The group also reported that PFC D1 receptor availability measured by [^{11}C]NNC112 was significantly upregulated in chronic ketamine users, although no significant relationships were found between PFC D1 receptor availability and performance on working memory tests (Narendran *et al*, 2005).

It has been discussed that these inconsistent results might stem from several factors including differences in radioligands, but our more recent PET study measuring cortical D1 receptors with both [^{11}C]SCH23390 and [^{11}C]NNC112 in the same schizophrenia population showed that prefrontal D1 receptors were decreased in chronic schizophrenia regardless of radioligands (Kosaka *et al*, 2010). Still, the reasons for these inconsistent results need to be clarified in the future. An inverted U-shaped response might account for working memory deficits in schizophrenia patients, whether D1 receptors in PFC are increased or decreased in patients.

The central profile of most antipsychotics is the D2 receptor blockade property. Antipsychotics are reasonably effective in ameliorating positive symptoms in schizophrenia. However, negative symptoms and cognitive impairments of schizophrenia are typically not responsive to antipsychotic therapy. This has led to the investigation of alternative agents for the treatment of cognitive impairments in schizophrenia, and a body of data from animal and human studies support the utility of the D1 agonist (Buchanan *et al*, 2007; Okubo *et al*, 1997*a*). However, the efficacy of D1 agonists on cognitive impairments has not so far been proven due to several practical issues of drug development. In addition to these issues, we need to taken into account the fact that schizophrenia is a heterogeneous disorder. D1 receptor density might be different according to the type of the disease, changeable even in a single patient according to its stage (prodromal phase, first episode phase, and chronic phase). The inverted U-shaped property of D1 receptor stimulation might lead to bidirectional effect of D1 agonist depending on the type or stage of schizophrenia. Anhedonia or blunted affect is one of the central features of negative symptoms. Some neuroimaging studies have suggested that reduced amygdala activation was associated with these symptoms (Dowd and Barch, 2010; Takahashi *et al*, 2004). Therefore, similarly to the strategy for cognitive impairment, D1 agonist might be useful for restoring amygdala activation, and consequently improve these negative symptoms.

Misestimating risk could lead to disadvantaged choices such as initiation of drug use/gambling and transition to regular drug use/gambling (Kreek *et al*, 2005). Our studies have shown that people with lower striatal D1 receptor availability tend to misestimate the weight of probabilities, and in particular, to overestimate low probabilities of winning gambles (risk seeking). This finding led us to the intuitive conjecture that D1 agonist, again, might be useful for easing misestimation of risk, and consequently beneficial for pathological gambling. However, on the contrary, clinical reports have indicated the association between dopamine agonist medication and the emergence of pathological gambling in Parkinson's disease patients (Gallagher *et al*, 2007). Although early reports implicated D3 receptor agonists as being most likely to induce pathological gambling in Parkinson's disease patients (Dodd *et al*, 2005), it has been reported that mixed D1/D2 receptor agonists can also promote pathological gambling (Lu *et al*, 2006). These clinical findings appear to challenge our prediction, but indeed they may not. Pathological gambling is a complex behavior, which has been related to failures in impulse control or response inhibition as observed in Parkinson's disease, but also to impaired decision making, including risky or ambiguous decision. Estimation of risk requires the latter high-level processing, and we would argue that this is related to striatal D1 receptor availability, leading to the following hypothesis: low-level striatal D1 receptor availability (which might in part be determined by genetic factors) is linked to a risk-seeking trait. The risk-seeking trait was reported to be linked to enhanced activation and DA release in the striatum during risk-seeking behavior (Leyton *et al*, 2002; St Onge and Floresco, 2009). Chronic exposure to unusually high release of DA by risk-seeking behavior might induce downregulation of D1 receptors (Moore *et al*, 1998; Yasuno *et al*, 2007). The further decrease in D1 receptor availability then leads to further risk seeking. Low-level striatal D1 receptor availability could therefore be a gateway to a vicious cycle, creating a predisposition to drug addiction and pathological gambling. Recently, circumstantial evidence to support this hypothesis has been reported. Martinez *et al* (2009), based on their PET study, suggested that reduced D1 receptor binding may be associated with an increased risk of relapse in cocaine addiction. Needless to say, this tentative hypothesis needs to be tested in future investigations, and we believe that understanding the molecular mechanism of extreme or impaired decision making will contribute to the assessment and prevention of drug and gambling addiction as well as the development of novel pharmacological therapies for these addictions. In conclusion, interdisciplinary approach combining molecular imaging techniques with cognitive neuroscience and clinical psychiatry will provide new perspectives for understanding the neurobiology of neuropsychiatric disorders and their innovative drug developments.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

References

Aalto S, Bruck A, Laine M, Nagren K, Rinne JO (2005) Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: a positron emission tomography study using the high-affinity dopamine D2 receptor ligand [11C]FLB 457. *J Neurosci* 25:2471–7

Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, Hwang DR, Keilp J, Kochan L, Van Heertum R, Gorman JM, Laruelle M (2002) Prefrontal dopamine D1 receptors and working memory in schizophrenia. *J Neurosci* 22:3708–19

Abi-Dargham A, Xu X, Thompson JL, Gil R, Kegeles LS, Urban NB, Narendran R, Hwang DR, Laruelle M, Slifstein M (2011) Increased prefrontal cortical D1 receptors in drug naive patients with schizophrenia: a PET study with [11C]NNC112. *J Psychopharmacol*; Epub ahead of print

Asan E (1998) The catecholaminergic innervation of the rat amygdala. *Adv Anat Embryol Cell Biol* 142:1–118

Bäckman L, Karlsson S, Fischer H, Karlsson P, Brehmer Y, Rieckmann A, MacDonald SW, Farde L, Nyberg L (2011) Dopamine D(1) receptors and age differences in brain activation during working memory. *Neurobiol Aging* 32:1849–56

Bissiere S, Humeau Y, Luthi A (2003) Dopamine gates LTP induction in lateral amygdala by suppressing feedforward inhibition. *Nat Neurosci* 6:587–92

Braver TS, Barch DM, Cohen JD (1999) Cognition and control in schizophrenia: a computational model of dopamine and prefrontal function. *Biol Psychiatry* 46:312–28

Buchanan RW, Freedman R, Javitt DC, Abi-Dargham A, Lieberman JA (2007) Recent advances in the development of novel pharmacological agents for the treatment of cognitive impairments in schizophrenia. *Schizophr Bull* 33:1120–30

Caille I, Dumartin B, Bloch B (1996) Ultrastructural localization of D1 dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervation. *Brain Res* 730:17–31

Camerer C, Loewenstein G (2004) Behavioral economics: past, present, future. In: *Advance in behavioral economics* (Camerer C, Loewenstein G, Rabin M, eds), Princeton, NJ: Princeton University Press pp 3–51

Castner SA, Goldman-Rakic PS (2004) Enhancement of working memory in aged monkeys by a sensitizing regimen of dopamine D1 receptor stimulation. *J Neurosci* 24:1446–50

Cools R, D'Esposito M (2011) Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol Psychiatry* 69:113–25

Corkin S (2001) Beware of frontal lobe deficits in hippocampal clothing. *Trends Cogn Sci* 5:321–3

de la Mora MP, Gallegos-Cari A, Arizmendi-Garcia Y, Marcellino D, Fuxe K (2010) Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: structural and functional analysis. *Prog Neurobiol* 90:198–216

De Martino B, Kumaran D, Seymour B, Dolan RJ (2006) Frames, biases, and rational decision-making in the human brain. *Science* 313:684–7

Dodd ML, Klos KJ, Bower JH, Geda YE, Josephs KA, Ahlskog JE (2005) Pathological gambling caused by drugs used to treat Parkinson disease. *Arch Neurol* 62:1377–81

Dowd EC, Barch DM (2010) Anhedonia and emotional experience in schizophrenia: neural and behavioral indicators. *Biol Psychiatry* 67:902–11

Dreher JC, Burnod Y (2002) An integrative theory of the phasic and tonic modes of dopamine modulation in the prefrontal cortex. *Neural Netw* 15:583–602

Dreyer JK, Herrik KF, Berg RW, Hounsgaard JD (2010) Influence of phasic and tonic dopamine release on receptor activation. *J Neurosci* 30:14273–83

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA* 98:6917–22

Ekelund J, Slifstein M, Narendran R, Guillin O, Belani H, Guo N, Hwang Y, Hwang D, Abi-Dargham A, Laruelle M (2007) *In vivo* DA D 1 receptor selectivity of NNC 112 and SCH 23390. *Mol Imaging Biol* 9:117–25

Floresco SB, Magyar O (2006) Mesocortical dopamine modulation of executive functions: beyond working memory. *Psychopharmacology (Berl)* 188:567–85

Fox C, Poldrack R (2009) Prospect theory and the brain. In: *Neuroeconomics* (Glimcher PW, Camerer C, Fehr E, Poldrack R, eds), London: Academic Press pp 145–74

Frank M, Scheres A, Sherman S (2007) Understanding decision-making deficits in neurological conditions: insights from models of natural action selection. *Philos Trans R Soc London B Biol Sci* 362:1641–54

Gallagher DA, O'Sullivan SS, Evans AH, Lees AJ, Schrag A (2007) Pathological gambling in Parkinson's disease: risk factors and differences from dopamine dysregulation. An analysis of published case series. *Mov Disord* 22:1757–63

Garris PA, Ciolkowski EL, Pastore P, Wightman R (1994) Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. *J Neurosci* 14: 6084–93

Glickstein SB, Hof PR, Schmauss C (2002) Mice lacking dopamine D2 and D3 receptors have spatial working memory deficits. *J Neurosci* 22:5619–29

Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS, Goldman D, Weinberger DR (2003) Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. *Arch Gen Psychiatry* 60:889–96

Goldman-Rakic PS, Muly III EC, Williams GV (2000) D(1) receptors in prefrontal cells and circuits. *Brain Res Brain Res Rev* 31:295–301

Goto Y, Grace AA (2008) Dopamine modulation of hippocampal prefrontal cortical interaction drives memory-guided behavior. *Cereb Cortex* 18:1407–14

Grace AA (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience* 41:1–24

Grace AA, Floresco SB, Goto Y, Lodge DJ (2007) Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends Neurosci* 30:220–7

Hall H, Sedvall G, Magnusson O, Kopp J, Halldin C, Farde L (1994) Distribution of D1- and D2-dopamine receptors, and dopamine and its metabolites in the human brain. *Neuropsychopharmacology* 11:245–56

Hariri AR, Mattay VS, Tessitore A, Fera F, Smith WG, Weinberger DR (2002) Dextroamphetamine modulates the response of the human amygdala. *Neuropsychopharmacology* 27:1036–40

Hsu M, Krajbich I, Zhao C, Camerer C (2009) Neural response to reward anticipation under risk is nonlinear in probabilities. *J Neurosci* 29:2231

Igarashi K, Oguni H, Osawa M, Awaya Y, Kato M, Mimura M, Kashima H (2002) Wisconsin card sorting test in children with temporal lobe epilepsy. *Brain Dev* 24: 174–8

Inglis FM, Moghaddam B (1999) Dopaminergic innervation of the amygdala is highly responsive to stress. *J Neurochem* 72:1088–94

Ito H, Takahashi H, Arakawa R, Takano H, Suhara T (2008) Normal database of dopaminergic neurotransmission system in human brain measured by positron emission tomography. *Neuroimage* 39:555–65

Kienast T, Hariri AR, Schlagenhauf F, Wrase J, Sterzer P, Buchholz HG, Smolka MN, Grunder G, Cumming P, Kumakura Y, Bartenstein P, Dolan RJ, Heinz A (2008) Dopamine in amygdala gates limbic processing of aversive stimuli in humans. *Nat Neurosci* 11:1381–2

Kosaka J, Takahashi H, Ito H, Takano A, Fujimura Y, Matsumoto R, Nozaki S, Yasuno F, Okubo Y, Kishimoto T, Suhata T (2010) Decreased binding of [11C] NNC112 and [11C] SCH23390 in patients with chronic schizophrenia. *Life Sci* 86:814–8

Kreek MJ, Nielsen DA, Butelman ER, LaForge KS (2005) Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. *Nat Neurosci* 8:1450–7

Kroner S, Rosenkranz JA, Grace AA, Barrionuevo G (2005) Dopamine modulates excitability of basolateral amygdala neurons *in vitro*. *J Neurophysiol* 93:1598–610

Laroche S, Davis S, Jay TM (2000) Plasticity at hippocampal to prefrontal cortex synapses: dual roles in working memory and consolidation. *Hippocampus* 10:438–46

LeDoux JE (2000) Emotion circuits in the brain. *Annu Rev Neurosci* 23:155–84

Leyton M, Boileau I, Benkelfat C, Diksic M, Baker G, Dagher A (2002) Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[11C] raclopride study in healthy men. *Neuropsychopharmacology* 27:1027

Lidow MS, Koh PO, Arnsten AF (2003) D1 dopamine receptors in the mouse prefrontal cortex: immunocytochemical and cognitive neuropharmacological analyses. *Synapse* 47:101–8

Lu C, Bharmal A, Suchowersky O (2006) Gambling and Parkinson disease. *Arch Neurol* 63:298

MacDonald SW, Cervenka S, Farde L, Nyberg L, Backman L (2009) Extrastriatal dopamine D2 receptor binding modulates intraindividual variability in episodic recognition and executive functioning. *Neuropsychologia* 47:2299–304

Marowsky A, Yanagawa Y, Obata K, Vogt KE (2005) A specialized subclass of interneurons mediates dopaminergic facilitation of amygdala function. *Neuron* 48:1025–37

Martinez D, Slifstein M, Narendran R, Foltin RW, Broft A, Hwang DR, Perez A, Abi-Dargham A, Fischman MW, Kleber HD, Laruelle M (2009) Dopamine D1 receptors in cocaine dependence measured with PET and the choice to self-administer cocaine. *Neuropsychopharmacology* 34:1774–82

Mattay VS, Goldberg TE, Fera F, Hariri AR, Tessitore A, Egan MF, Kolachana B, Callicott JH, Weinberger DR (2003) Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc Natl Acad Sci USA* 100: 6186–91

McDowell S, Whyte J, D'Esposito M (1998) Differential effect of a dopaminergic agonist on prefrontal function in traumatic brain injury patients. *Brain* 121:1155–64

McNab F, Varrone A, Farde L, Jucaite A, Bystritsky P, Forssberg H, Klingberg T (2009) Changes in cortical dopamine D1 receptor binding associated with cognitive training. *Science* 323:800–2

Mehta MA, Sahakian BJ, McKenna PJ, Robbins TW (1999) Systemic sulpiride in young adult volunteers simulates the profile of cognitive deficits in Parkinson's disease. *Psychopharmacology (Berl)* 146:162–74

Moore R, Vinsant S, Nader M, Porrino L, Friedman D (1998) Effect of cocaine self-administration on dopamine D2 receptors in rhesus monkeys. *Synapse* 30:88–96

Müller U, von Cramon DY, Pollmann S (1998) D1- versus D2-receptor modulation of visuospatial working memory in humans. *J Neurosci* 18:2720–8

Narendran R, Frankle WG, Keefe R, Gil R, Martinez D, Slifstein M, Kegeles LS, Talbot PS, Huang Y, Hwang DR, Khenissi L, Cooper TB, Laruelle M, Abi-Dargham A (2005) Altered prefrontal dopaminergic function in chronic recreational ketamine users. *Am J Psychiatry* 162:2352–9

Okubo Y, Suhara T, Sudo Y, Toru M (1997*a*) Possible role of dopamine D1 receptors in schizophrenia. *Mol Psychiatry* 2:291–2

Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O, Someya Y, Sassa T, Sudo Y, Matsushima E, Iyo M, Tateno Y, Toru M (1997*b*) Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 385:634–6

Pezze MA, Feldon J (2004) Mesolimbic dopaminergic pathways in fear conditioning. *Prog Neurobiol* 74: 301–20

Phillips AG, Ahn S, Floresco SB (2004) Magnitude of dopamine release in medial prefrontal cortex predicts accuracy of memory on a delayed response task. *J Neurosci* 24:547–53

Prelec D (1998) The probability weighting function. *Econometrica* 66:497–527

Rangel A, Camerer C, Montague PR (2008) A framework for studying the neurobiology of value-based decision making. *Nat Rev Neurosci* 9:545–56

Richfield EK, Penney JB, Young AB (1989) Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. *Neuroscience* 30:767–77

Rosenkranz JA, Grace AA (1999) Modulation of basolateral amygdala neuronal firing and afferent drive by dopamine receptor activation *in vivo*. *J Neurosci* 19:11027–39

Rosenkranz JA, Grace AA (2002) Cellular mechanisms of infralimbic and prelimbic prefrontal cortical inhibition and dopaminergic modulation of basolateral amygdala neurons *in vivo*. *J Neurosci* 22:324–37

Rottenstreich Y, Hsee CK (2001) Money, kisses, and electric shocks: on the affective psychology of risk. *Psychol Sci* 12:185–90

Royer S, Martina M, Pare D (1999) An inhibitory interface gates impulse traffic between the input and output stations of the amygdala. *J Neurosci* 19:10575–83

Sawaguchi T, Goldman-Rakic PS (1994) The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J Neurophysiol* 71:515–28

Schultz W (2007*a*) Multiple dopamine functions at different time courses. *Annu Rev Neurosci* 30:259–88

Schultz W (2007*b*) Behavioral dopamine signals. *Trends Neurosci* 30:203–10

Seamans JK, Floresco SB, Phillips AG (1998) D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J Neurosci* 18:1613–21

Seamans JK, Yang CR (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol* 74:1–58

Slifstein M, Kegeles LS, Gonzales R, Frankle WG, Xu X, Laruelle M, Abi-Dargham A (2007) [11C]NNC 112 selectivity for dopamine D1 and serotonin 5-HT(2A) receptors: a PET study in healthy human subjects. *J Cereb Blood Flow Metab* 27:1733–41

Slifstein M, Kolachana B, Simpson EH, Tabares P, Cheng B, Duvall M, Frankle WG, Weinberger DR, Laruelle M, Abi-Dargham A (2008) COMT genotype predicts cortical-limbic D1 receptor availability measured with [11C]NNC112 and PET. *Mol Psychiatry* 13:821–7

Smiley JF, Levey AI, Ciliax BJ, Goldman-Rakic PS (1994) D1 dopamine receptor immunoreactivity in human and monkey cerebral cortex: predominant and extrasynaptic localization in dendritic spines. *Proc Natl Acad Sci USA* 91:5720–4

Steeves TD, Miyasaki J, Zurowski M, Lang AE, Pellecchia G, Van Eimeren T, Rusjan P, Houle S, Strafella AP (2009) Increased striatal dopamine release in Parkinsonian patients with pathological gambling: a [11C] raclopride PET study. *Brain* 132:1376–85

St Onge JR, Floresco SB (2009) Dopaminergic modulation of risk-based decision making. *Neuropsychopharmacology* 34:681–97

Suhara T, Sudo Y, Okauchi T, Maeda J, Kawabe K, Suzuki K, Okubo Y, Nakashima Y, Ito H, Tanada S, Halldin C, Farde L (1999) Extrastriatal dopamine D2 receptor density and affinity in the human brain measured by 3D PET. *Int J Neuropsychopharmcol* 2:73–82

Takahashi H, Kato M, Hayashi M, Okubo Y, Takano A, Ito H, Suhara T (2007) Memory and frontal lobe functions; possible relations with dopamine D2 receptors in the hippocampus. *Neuroimage* 34:1643–9

Takahashi H, Kato M, Takano H, Arakawa R, Okumura M, Otsuka T, Kodaka F, Hayashi M, Okubo Y, Ito H, Suhara

T (2008) Differential contributions of prefrontal and hippocampal dopamine D(1) and D(2) receptors in human cognitive functions. *J Neurosci* 28:12032–8

Takahashi H, Koeda M, Oda K, Matsuda T, Matsushima E, Matsuura M, Asai K, Okubo Y (2004) An fMRI study of differential neural response to affective pictures in schizophrenia. *Neuroimage* 22:1247–54

Takahashi H, Matsui H, Camerer C, Takano H, Kodaka F, Ideno T, Okubo S, Takemura K, Arakawa R, Eguchi Y, Murai T, Okubo Y, Kato M, Ito H, Suhara T (2010*a*) Dopamine D receptors and nonlinear probability weighting in risky choice. *J Neurosci* 30:16567–72

Takahashi H, Takano H, Kodaka F, Arakawa R, Yamada M, Otsuka T, Hirano Y, Kikyo H, Okubo Y, Kato M, Obata T, Ito H, Suhara T (2010*b*) Contribution of dopamine D1 and D2 receptors to amygdala activity in human. *J Neurosci* 30:3043–7

Takahashi H, Yahata N, Koeda M, Takano A, Asai K, Suhara T, Okubo Y (2005) Effects of dopaminergic and serotonergic manipulation on emotional processing: a pharmacological fMRI study. *Neuroimage* 27:991–1001

Tessitore A, Hariri AR, Fera F, Smith WG, Chase TN, Hyde TM, Weinberger DR, Mattay VS (2002) Dopamine modulates the response of the human amygdala: a study in Parkinson's disease. *J Neurosci* 22:9099–103

Thierry AM, Gioanni Y, Degenetais E, Glowinski J (2000) Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. *Hippocampus* 10:411–9

Tom S, Fox C, Trepel C, Poldrack R (2007) The neural basis of loss aversion in decision-making under risk. *Science* 315:515

Trepel C, Fox C, Poldrack R (2005) Prospect theory on the brain? Toward a cognitive neuroscience of decision under risk. *Cogn Brain Res* 23:34–50

Tseng KY, Lewis BL, Lipska BK, O'Donnell P (2007) Post-pubertal disruption of medial prefrontal cortical dopamine-glutamate interactions in a developmental animal model of schizophrenia. *Biol Psychiatry* 62:730–8

Tversky A, Kahneman D (1992) Advances in prospect theory: cumulative representation of uncertainty. *J Risk Uncertain* 5:297–323

Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF (2007) Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat Neurosci* 10:376–84

Williams GV, Castner SA (2006) Under the curve: critical issues for elucidating D1 receptor function in working memory. *Neuroscience* 139:263–76

Williams-Gray CH, Hampshire A, Robbins TW, Owen AM, Barker RA (2007) Catechol O-methyltransferase Val158-Met genotype influences frontoparietal activity during planning in patients with Parkinson's disease. *J Neurosci* 27:4832–8

Yamamoto R, Ueta Y, Kato N (2007) Dopamine induces a slow after depolarization in lateral amygdala neurons. *J Neurophysiol* 98:984–92

Yasuno F, Ota M, Ando K, Ando T, Maeda J, Ichimiya T, Takano A, Doronbekov TK, Fujimura Y, Nozaki S, Suhara T (2007) Role of ventral striatal dopamine D1 receptor in cigarette craving. *Biol Psychiatry* 61:1252–9

Zack M, Poulos C (2004) Amphetamine primes motivation to gamble and gambling-related semantic networks in problem gamblers. *Neuropsychopharmacology* 29:195–207

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Chronic Fluoxetine Selectively Upregulates Dopamine D_1-Like Receptors in the Hippocampus

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The dentate gyrus of the hippocampus has been implicated in mechanisms of action of selective serotonin reuptake inhibitors (SSRIs). We have recently demonstrated that the SSRI fluoxetine can reverse the state of maturation of the adult dentate granule cells and enhances serotonin 5-HT_4 receptor-mediated synaptic potentiation at the synapses formed by their mossy fiber axons. Here, we show that fluoxetine can induce long-lasting enhancement of dopaminergic modulation at the mossy fiber synapse. Synaptic responses arising from the mossy fiber-CA3 pyramidal cell synapse were recorded using acute mouse hippocampal slices. Dopamine potentiates mossy fiber synaptic transmission by activating D_1-like receptors. Chronic fluoxetine treatment induced a prominent increase in the magnitude of dopamine-induced synaptic potentiation, and this effect was maintained at least up to 1 month after withdrawal of fluoxetine. Quantitative autoradiography revealed that binding of the D_1-like receptor ligand [^{3}H]SCH23390 was selectively increased in the dentate gyrus and along the mossy fiber in fluoxetine-treated mice. However, binding of the 5-HT_4 receptor ligand [^{3}H]GR113808 was not significantly changed. These results suggest that chronic fluoxetine enhanced the dopaminergic modulation at least in part by upregulating expression of D_1-like receptors, while the enhanced serotonergic modulation may be mediated by modifications of downstream signaling pathways. These enhanced monoaminergic modulations would greatly increase excitatory drive to the hippocampal circuit through the dentate gyrus. The highly localized upregulation of D_1-like receptors further supports the importance of the dentate gyrus in the mechanism of action of SSRIs.

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INTRODUCTION

The central serotonergic system is the crucial target for pharmacological treatments of psychiatric disorders (Vaswani *et al*, 2003; Meltzer and Massey, 2011). Drugs that can raise extracellular serotonin levels such as selective serotonin reuptake inhibitors (SSRIs) have been widely used to treat mood and anxiety disorders (Vaswani *et al*, 2003). However, cellular mechanisms underlying both therapeutic and adverse effects of SSRIs have not been fully understood. The dentate gyrus of the hippocampus has been implicated in behavioral effects of SSRIs and other antidepressant drugs in experimental animals (Adachi *et al*, 2008; Kobayashi *et al*, 2011a; Miyamoto *et al*, 2011; Sahay and Hen, 2007; Santarelli *et al*, 2003). We have recently shown that the SSRI fluoxetine greatly changes serotonergic modulation at the synapse between the mossy fiber, the sole output of the dentate granule cell, and CA3 pyramidal cells (Kobayashi *et al*, 2008, 2010). At the mossy fiber-CA3 synapse, serotonin induces robust synaptic potentiation and small depression by activating 5-HT_4 and 5-HT_{1A} receptors, respectively (Kobayashi *et al*, 2008, 2010), and chronic fluoxetine administered at a relatively high dose causes marked enhancement of serotonin-induced synaptic potentiation (Kobayashi *et al*, 2010). We have also shown that fluoxetine reduces strong synaptic facilitation at the mossy fiber synapse to a juvenile level via a process characterized as 'dematuration' of mature granule cells (Kobayashi *et al*, 2010). In mice lacking the 5-HT_4 receptor, the serotonin-induced synaptic potentiation at the mossy fiber synapse was abolished and dematuration of granule cells was attenuated (Kobayashi *et al*, 2010), suggesting a critical role of the 5-HT_4 receptor in cellular effects of fluoxetine in the hippocampus. As the mossy fiber has an essential role in regulating excitability and associative synaptic plasticity in the CA3 pyramidal cells (Kobayashi and Poo, 2004), the fluoxetine-induced alteration of mossy fiber synaptic

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transmission is likely to have a substantial impact on functioning of the hippocampal circuit. Indeed, chronic fluoxetine caused significant changes in some forms of hippocampus-dependent behaviors, such as locomotor activity and anxiety-related behaviors, and these behavioral effects of fluoxetine were reduced in the 5-HT_4 receptor-deficient mice (Kobayashi *et al*, 2011a). Furthermore, the fluoxetine-induced reduction of mossy fiber synaptic facilitation significantly correlated with the behavioral change caused by fluoxetine in individual mice (Kobayashi *et al*, 2011a). Stress, which is generally thought to precipitate psychiatric disorders including depression, changes the structure and functions of the mossy fiber synapse (Chen *et al*, 2010; Kobayashi, 2010; Magariños *et al*, 1997). These lines of evidence suggest that the mossy fiber synapse could be an important target for SSRIs and other antidepressant drugs (Kobayashi, 2009, 2010).

The central dopaminergic system has also been suggested to be an important target for the treatment of psychiatric disorders. Dopamine has been implicated in the pathophysiology of mood disorders (Suhara *et al*, 1992) and mechanisms of action of antidepressant drugs including SSRIs (D'Aquila *et al*, 2000). Dopamine can potentiate the mossy fiber synaptic transmission, and this effect is associated with activity of mice in novel environments (Kobayashi *et al*, 2006). The dopamine-induced synaptic potentiation at the mossy fiber synapse is mediated by D_1-like receptors (Kobayashi and Suzuki, 2007). D_1-like receptors are involved in behavioral effects of antidepressants in animal models of depression (D'Aquila *et al*, 1994; Gambarana *et al*, 1995; Sampson *et al*, 1991). Given the potential involvement of the mossy fiber synapse in mechanisms of action of antidepressants, the dopaminergic modulation at the mossy fiber synapse may also be affected by antidepressant treatments. To address this issue, in the present study, we examined effects of dopamine on the mossy fiber synaptic transmission in hippocampal slices prepared from mice chronically treated with fluoxetine, and found that fluoxetine causes prominent long-lasting enhancement of the dopamine-induced synaptic potentiation. The involvement of the serotonergic system in this effect of fluoxetine was examined by using mice with lesions in the central serotonergic neurons and mice deficient for the 5-HT_4 receptor, in both of which the effectiveness of fluoxetine in inducing the granule cell dematuration has been shown to be greatly reduced in the same treatment regimen (Kobayashi *et al*, 2010). We further investigated the possibility that the enhanced monoaminergic modulation at the mossy fiber synapse is mediated by changes in expression levels of monoamine receptors that contribute to the synaptic modulation by quantitative autoradiography.

MATERIALS AND METHODS

Drug Treatment

Male C57BL/6J mice were singly housed from the age of 8 weeks in the institutional standard condition (14 : 10 light/dark cycle; lights on at 0600 h through 2800 h) at 23 ± 1 °C with food and water available ad libitum. Following 1 week of acclimation, fluoxetine hydrochloride (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in the drinking water and orally applied as described (Kobayashi *et al*, 2010, 2011a). As fluoxetine reduced water consumption, saccharin (0.2%) was included to keep a water intake comparable to the baseline. Concentrations of fluoxetine in the drinking water were determined for individual mice everyday based on the water consumption during preceding 24 h and the body weight measured every other day. Control mice (CNT) were given water with or without saccharin, and all data were pooled. Paroxetine (PAR) hydrochloride (Toronto Research Chemicals, North York, Ontario, Canada) was administered in the same way as fluoxetine. The 5-HT_4 receptor mutant mice (strain name: B6.129P2-Htr4 < tm1Dgen > /J) backcrossed to the C57BL/6J background were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Male homozygous mutant mice and their wild-type littermates from heterozygous mating were treated with fluoxetine in the same way and used for electrophysiological experiments. We shared some data from control groups of both wild-type and mutant mice with our previous study (Kobayashi *et al*, 2010). All procedures were approved by the Animal Care and Use Committee of Nippon Medical School.

Electrophysiology

Mice were decapitated under deep halothane anesthesia and hippocampi were isolated. Transverse hippocampal slices (380 μm) were cut using a tissue slicer in ice-cold saline as described (Kobayashi *et al*, 2010) and maintained in a humidified interface holding chamber at room temperature (24–27°C) before recording. Electrophysiological recordings were made in a submersion-type chamber maintained at 27.0–27.5°C and superfused at 2 ml/min with saline composed of (in mM): NaCl 125; KCl, 2.5; NaH_2PO_4, 1.0; $NaHCO_3$, 26.2; glucose, 11; $CaCl_2$, 2.5; $MgCl_2$, 1.3 (equilibrated with 95% O_2/5% CO_2). Electrical stimulation was delivered to the dentate granule cell layer, and field excitatory postsynaptic potentials (fEPSPs) arising from the mossy fiber synapses were recorded from the stratum lucidum of CA3 using a glass pipette filled with 2 M NaCl. The amplitude of fEPSPs was measured on analysis as described (Kobayashi and Suzuki, 2007). A criterion used to identify the mossy fiber input was > 85% block of the fEPSP amplitude by an agonist of group II metabotropic glutamate receptors, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV, 1 μM) (Tocris Bioscience, Bristol, UK). Single electrical stimulation was delivered at a frequency of 0.05 Hz for baseline recordings. Dopamine hydrochloride was purchased from Wako Pure Chemical Industries. SCH23390 was from Tocris Bioscience. All recordings were made using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA), filtered at 2 kHz and stored in a personal computer via an interface (digitized at 5–10 kHz).

Autoradiography

Mice were killed by decapitation under deep halothane anesthesia and their brains were quickly removed. One hemisphere of the brain was frozen with powdered dry ice and cut into 20-μm-thick coronal sections with a HM560

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cryotome (Carl Zeiss, Oberkochen, Germany). The other hemisphere was used for the electrophysiological experiment. The sections were mounted on slide glass (Matsunami Glass, Osaka, Japan) and stored at −80°C until use. All radiochemicals were purchased from GE Healthcare Bio-Sciences (Piscataway, NJ). Specific binding of the dopamine D_1-like receptor ligand [^{3}H]SCH233090 (1 nM) to the brain slice was analyzed by autoradiography as described (Mansour *et al*, 1990). The brain slices were pre-incubated for 15 min in 50 mM Tris-HCl buffer (pH 7.4, 25°C) containing 120 mM NaCl, 5 mM KCl, and 1 mM $MgCl_2$. The samples were then incubated at room temperature for 1 h in the same buffer containing 1 μM ketanserin and 1 nM [^{3}H]SCH23390. Nonspecific binding was assessed in the presence of 1 μM of cis-flupentixol. Specific binding of serotonin 5-HT_4 receptor ligand [^{3}H] GR113808 was analyzed as described (López-Giménez *et al*, 2002). The brain slices were pre-incubated for 20 min in 50 mM HEPES buffer (pH 7.4, 25°C) containing 10 μM pargyline and 0.01% ascorbic acid. The samples were then incubated at room temperature for 1 h in the same buffer containing 0.1 nM [^{3}H]GR113808. Nonspecific binding was assessed in the presence of 100 μM of serotonin. Specific binding of the serotonin 5-HT_{1A} receptor ligand [^{3}H]8-OHDPAT was analyzed as described (Vergé *et al*, 1986). The brain slices were pre-incubated for 30 min in 170 mM Tris-HCl buffer (pH 7.4, 25°C) containing 4 mM $CaCl_2$, 10 μM pargyline, and 0.01% ascorbic acid. The samples were then incubated at room temperature for 1 h in the same buffer containing 2 nM [^{3}H]8-OHDPAT. Nonspecific binding was assessed in the presence of 100 μM of serotonin. Following the incubation, the samples were rinsed with ice-cold buffer, and desalted with ice-cold distilled water. The slices were subsequently dried under warm blowing air and exposed to a BAS-TR2025 imaging plate (Fuji Film, Tokyo, Japan). Exposure time for [^{3}H]SCH233090, [^{3}H]8-OHDPAT, and [^{3}H]GR113808 was 5, 7, and 28 days, respectively. The imaging plate was subsequently scanned with a BAS5000 system (Fuji Film). Regions of interest (ROIs) were defined on the images using a Multi Gauge software (Fuji Film), and densitometric assay for each ROI was performed using autoradiographic [^{3}H]micro-scales (GE Healthcare Bio-Sciences).

5,7-Dihydroxytryptamine (DHT) Lesion

To lesion the central serotonergic system, the serotonergic neurotoxin DHT(MP Biomedicals, OH, USA) was intracerebroventricularly injected as previously described (Kobayashi *et al*, 2010). Briefly, mice were anesthetized with pentobarbital (60 mg/kg i.p.) and were also injected with desipramine (25 mg/kg i.p.) to block the uptake of DHT by noradrenergic terminals. A total of 100 μg of DHT was dissolved in 10 μl of 0.9% sterile saline supplemented with 0.1% ascorbic acid, and slowly infused into the lateral cerebral ventricle. After the injections, mice were allowed to recover for 8–11 days and then treated with fluoxetine, as above.

Statistics

All data are presented as mean ± SEM. The number of data (n) represents the number of mice unless otherwise specified. Statistical tests were performed using GraphPad Prism version 3.03 for Windows (GraphPad Software, San Diego, CA, USA) with the significance level $p<0.05$. The two-tailed t test was used to compare two groups, and the Bonferroni's multiple comparison test or Dunn's multiple comparison test was used to compare three groups or more.

RESULTS

Fluoxetine Enhances Dopamine-Induced Potentiation at Mossy Fiber Synapse

We first examined effects of chronic fluoxetine treatment on the dopaminergic modulation at the mossy fiber-CA3 synapse. Mice were treated with fluoxetine at a dose of 22 mg/kg per day for 4 weeks, a regimen sufficient for the induction of the granule cell dematuration and enhancement of the serotonergic modulation (Kobayashi *et al*, 2010). Using acute hippocampal slices, fEPSPs arising from the mossy fiber synapses were recorded. Bath-applied dopamine (10 μM) induced robust potentiation of fEPSPs (to 171 ± 7% of baseline, $n=8$) as in previous studies (Kobayashi *et al*, 2006; Kobayashi and Suzuki, 2007). In fluoxetine-treated mice (FLX), the magnitude of dopamine-induced potentiation was strongly enhanced (to 348 ± 28% of baseline, $n=11$, $p<0.001$) (Figures 1a and b). At 14 mg/kg per day, fluoxetine had no significant effects on the dopamine-induced potentiation (Figure 1b). The effect of fluoxetine at 22 mg/kg per day was already evident after 2 weeks of treatment (Figure 1b) and could be observed at least up to 4 weeks after withdrawal of fluoxetine (Figure 1c). Chronic treatment with another SSRI PAR similarly enhanced the effect of dopamine (Figure 1b). As reported previously (Kobayashi and Suzuki, 2007), dopamine increased the amplitude of the presynaptic fiber volley component of the field potentials. Although this effect was also slightly augmented in the FLX, there was no statistically significant difference between two groups (control: 118 ± 3% of baseline; fluoxetine: 125 ± 6% of baseline; $p=0.2810$). The potentiating effect of dopamine at the mossy fiber synapse is mediated by D_1-like receptors (Kobayashi and Suzuki, 2007). In the FLX, the effect of dopamine was nearly completely suppressed when slices were pretreated with the D_1-like receptor antagonist SCH23390 (30 nM) (control slice: to 304 ± 29% of baseline, $n=6$ slices; pretreated slice: to 105 ± 2% of baseline, $n=6$ slices; $p=0.001$). These results indicate that chronic fluoxetine can induce long-lasting enhancement of the potentiating effect of dopamine mediated by D_1-like receptors at the hippocampal mossy fiber-CA3 synapse.

Requirement of Serotonergic System for Fluoxetine-Induced Enhancement of Dopaminergic Modulation

As the primary target of fluoxetine is the serotonin transporter, we examined the involvement of the serotonergic system in the fluoxetine-induced enhancement of the dopaminergic modulation. To lesion the central serotonergic system, mice were intracerebroventricularly injected with the serotonergic neurotoxin DHT. In vehicle-treated mice, chronic fluoxetine induced robust enhancement of dopamine-induced potentiation as in normal mice

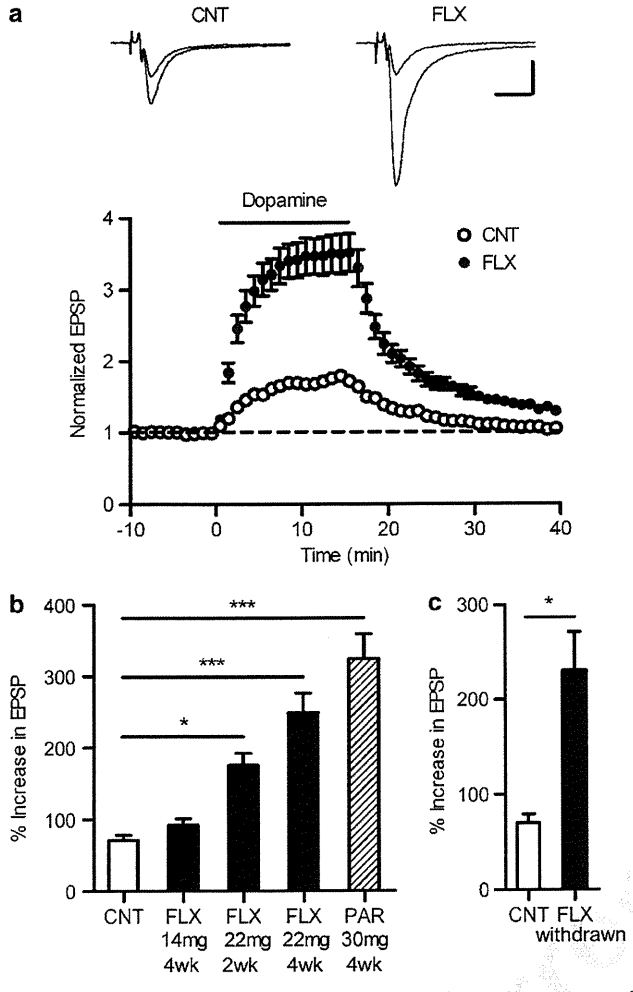

Figure 1 Chronic fluoxetine induces long-lasting enhancement of dopaminergic synaptic modulation. (a) Bath-applied dopamine induced reversible potentiation of mossy fiber synaptic transmission. The magnitude of potentiation was clearly increased in fluoxetine-treated mice (FLX) as compared with control mice (CNT). Sample traces show averages of 15 consecutive field excitatory postsynaptic potentials (fEPSPs) before and during dopamine application. Scale bar: 10 ms, 0.2 mV. (b) Effects of fluoxetine and paroxetine (PAR) on dopaminergic synaptic modulation. Dopamine-induced potentiation was significantly increased after 2 weeks ($n=5$, $p<0.05$) and 4 weeks ($n=11$, $p<0.001$, Bonferroni's multiple comparison test) of fluoxetine treatments at 22 mg/kg per day and 4 weeks of PAR treatment at 30 mg/kg per day ($n=4$, $p<0.001$), but not after 4 weeks of fluoxetine treatment at 14 mg/kg per day ($n=8$). (c) Dopamine-induced potentiation remained enhanced for at least 1 month after withdrawal of fluoxetine ($n=6$ each, $p=0.0133$).

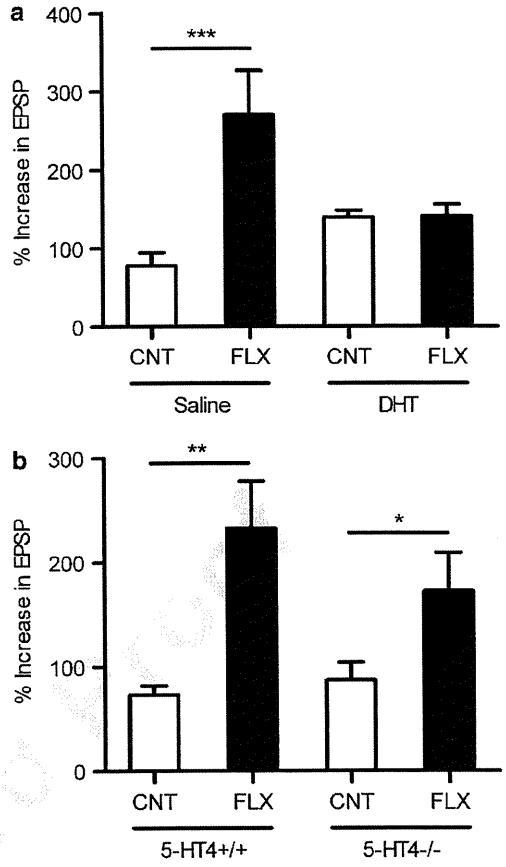

Figure 2 Serotonin dependence of fluoxetine-induced enhancement of dopaminergic modulation. (a) Chronic fluoxetine significantly increased the magnitude of dopamine-induced potentiation in saline-injected mice (control mice (CNT): $n=6$, fluoxetine-treated mice (FLX): $n=3$, $p<0.001$, Bonferroni's multiple comparison test), but not in 5,7-dihydroxytryptamine (DHT)-injected mice (CNT: $n=5$, FLX: $n=4$). There was no significant difference between saline- and DHT-injected control groups. (b) Chronic fluoxetine significantly increased the magnitude of dopamine-induced potentiation in both wild-type (5-HT_4+/+, CNT: $n=6$, FLX: $n=8$, $p<0.01$) and mutant mice (5-HT_4-/-, CNT: $n=10$, FLX: $n=9$, $p<0.05$, Dunn's multiple comparison test).

(Figure 2a). In DHT-treated mice, dopamine-induced potentiation was slightly increased in magnitude in the control condition, and chronic fluoxetine did not affect the magnitude of potentiation (Figure 2a), suggesting that the integrity of the serotonergic system is required for the effect of fluoxetine on the dopaminergic modulation. We have previously shown that the serotonin 5-HT_4 receptor has an important role in denaturation of the granule cell by fluoxetine (Kobayashi *et al*, 2010). We examined whether this receptor also contributes to the enhancement of dopamine-induced potentiation using mice lacking the 5-HT_4 receptor. In both wild-type and mutant mice, fluoxetine caused significant enhancement of dopamine-induced potentiation (Figure 2b). Therefore, the 5-HT_4 receptor is not essential for the enhancement of the potentiating effect of dopamine.

Fluoxetine Increases D_1-like Receptor Ligand Binding in the Dentate Gyrus and CA3

As shown above, the dopamine-induced synaptic potentiation in the FLX required activation of D_1-like receptors as in normal mice. The prominent enhancement of the effect of dopamine by fluoxetine might be mediated by an increase in expression of the D_1-like receptors at the mossy fiber synapse. To test this possibility, we examined binding of the D_1-like receptor ligand [^{3}H]SCH23390 by quantitative autoradiography. In CNT, strong [^{3}H]SCH23390 binding was seen in the striatum, and relatively weak binding was

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detected in the hippocampus (Figure 3a). Chronic fluoxetine significantly increased the [^{3}H]SCH23390 binding in the dentate gyrus and CA3 region (Figures 3a and b). After the treatment, the signal was visible along the mossy fiber pathway (Figure 3a). There was no significant change in the binding in the hippocampal CA1 region and other brain regions including striatum (Figure 3b). These results suggest that chronic fluoxetine caused selective upregulation of the dopamine D_1-like receptor expression in the dentate gyrus and along the mossy fiber pathway.

In order to test a possible involvement of changes in serotonin receptor expression in the fluoxetine-induced enhancement of the serotonergic modulation, we examined binding of the 5-HT_4 receptor-specific ligand [^{3}H]GR113808. Chronic fluoxetine caused an overall decrease in the [^{3}H]GR113808 binding (Figure 4a), which is consistent with previous results (Licht *et al*, 2009; Vidal *et al*, 2009). The decrease was evident in the striatum, amygdala, and substantia nigra, but did not reach the statistical significance in the hippocampus (Figure 4b). In addition to 5-HT_4 receptor-mediated synaptic potentiation, serotonin can cause weak synaptic inhibition via activation of the 5-HT_{1A} receptor at the mossy fiber synapse (Kobayashi *et al*, 2008). We also examined a possible change in expression of the 5-HT_{1A} receptor at the mossy fiber synapse by analyzing binding of the 5-HT_{1A} ligand [^{3}H]8-OHDPAT. Although fluoxetine significantly decreased the [^{3}H]8-OHDPAT binding in the CA1 region, it had no significant effect on the binding in the dentate gyrus or CA3 region (Figure 5). These results suggest that chronic fluoxetine had no significant effects on the expression of these serotonin receptors at the mossy fiber synapse.

DISCUSSION

The present study has demonstrated that chronic fluoxetine treatment causes long-lasting strong enhancement of dopamine D_1-like receptor-dependent synaptic potentiation at the hippocampal mossy fiber synapse and also selectively upregulates the binding of the D_1-like receptor ligand in the dentate gyrus and along the mossy fiber pathway. These results suggest that the enhanced dopaminergic synaptic modulation caused by fluoxetine is at least in part mediated by increased expression levels of D_1-like receptors. On the other hand, there were no significant changes in the binding of specific ligands for serotonin receptors that are involved in the modulation of mossy fiber synaptic transmission. Therefore, the enhanced serotonergic modulation by fluoxetine shown previously is likely mediated by modifications of receptor functioning or intracellular pathways downstream of the receptor activation.

Dopamine has been implicated in neuronal bases of action of antidepressant drugs including SSRIs (D'Aquila *et al*, 1994, 2000; Gambarana *et al*, 1995; Sampson *et al*, 1991). Consistent with our finding, chronic fluoxetine has been shown to increase mRNA levels of the D_1 receptor in the hippocampus (Miller *et al*, 2008). Increased D_1 receptor

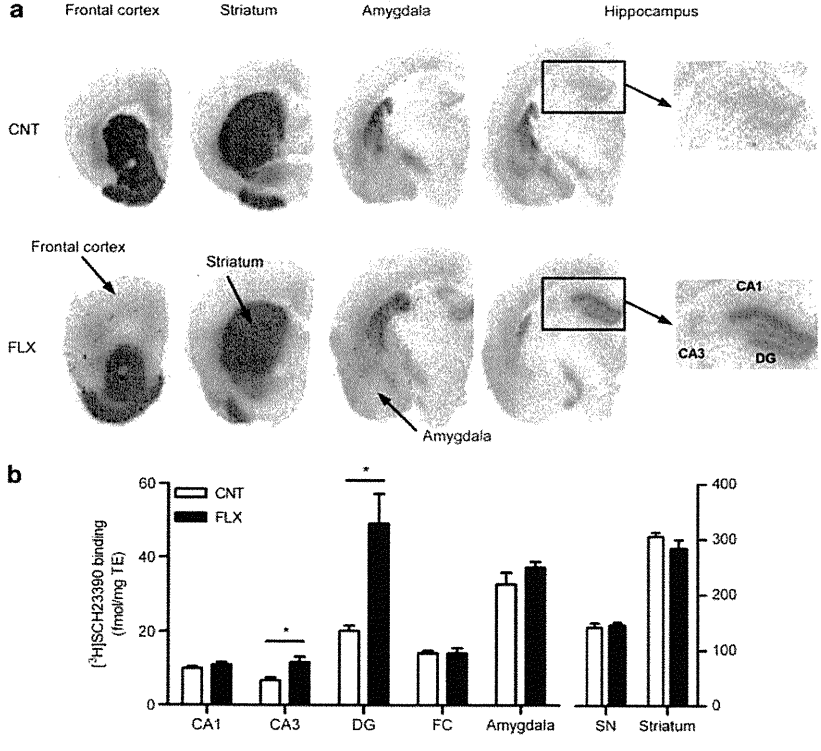

Figure 3 Selective increase in D_1-like ligand binding in dentate gyrus and CA3 in fluoxetine-treated mice (FLX). (a) Representative autoradiograms of [^{3}H]SCH23390 binding at four different section levels. (b) Summary data showing significant increases in [^{3}H]SCH23390 binding after fluoxetine treatment in CA3 ($p = 0.019$) and dentate gyrus ($p = 0.0121$) (control mice (CNT): $n = 6$, FLX: $n = 7$). DG, dentate gyrus; FC, frontal cortex; SN, substantia nigra.

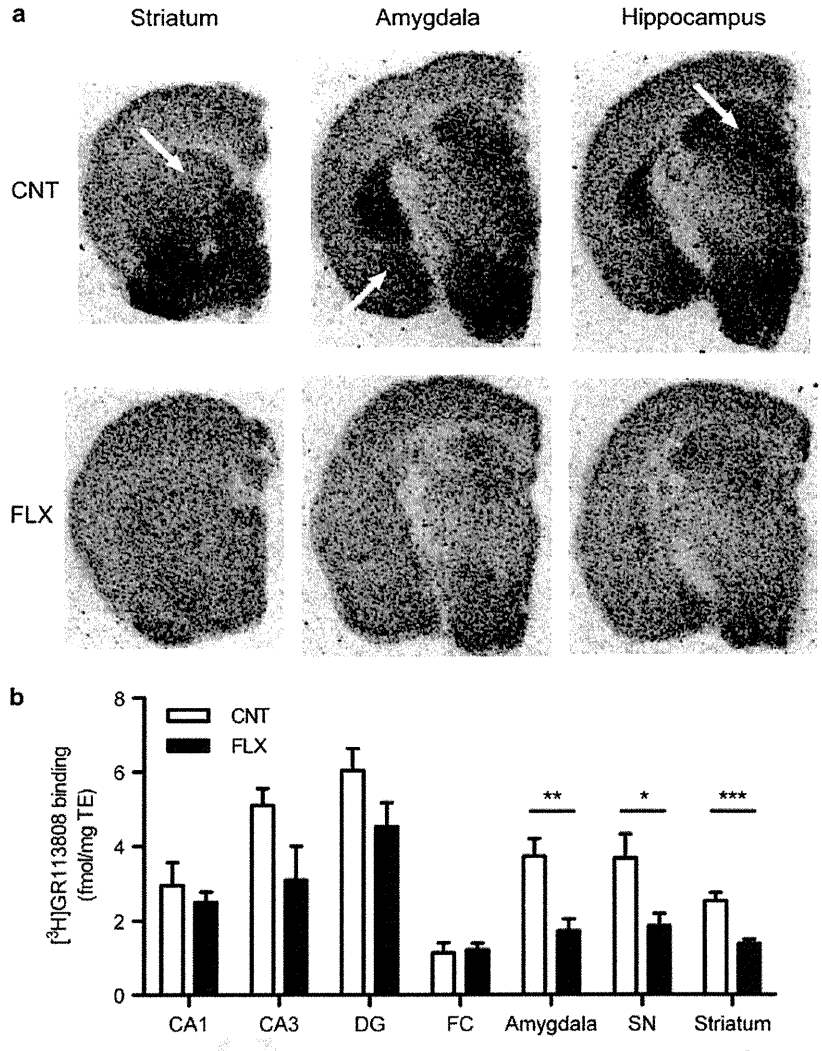

Figure 4 Reduced 5-HT_4 ligand binding in fluoxetine-treated mice (FLX). (a) Representative autoradiograms of [^{3}H]GR113808 binding in the striatum, amygdala and hippocampus (indicated by arrows). (b) Fluoxetine significantly reduced [^{3}H]GR113808 binding in amygdala ($p = 0.0043$), striatum ($p = 0.0005$) and substantia nigra ($p = 0.0239$) (control mice (CNT): $n = 6$, FLX: $n = 7$).

mRNA has also been demonstrated in the striatum and nucleus accumbens after repeated treatments with the SSRI sertraline (Huzarska *et al*, 2006). However, other studies have reported no effect of SSRIs on D_1 mRNA levels in the striatum and nucleus accumbens (Ainsworth *et al*, 1998; Dziedzicka-Wasylewska *et al*, 1997). In membrane preparations of the striatum, D_1-like ligand binding did not change (Deslandes *et al*, 2002) or decreased after SSRI treatments (Klimek and Nielsen, 1987). Decreased D_1-like ligand binding has also been demonstrated in the membrane preparation of the limbic system (Klimek and Nielsen, 1987). In the present study, the D_1-like ligand binding was selectively increased in the dentate gyrus and hippocampal CA3 region. This highly localized hippocampus-specific increase in D_1-like ligand binding is in agreement with the lack of an increase in D_1 mRNA levels or D_1-like ligand binding in the brain regions other than the hippocampus in most previous studies. We have previously demonstrated a similar marked increase in D_1-like ligand binding that is restricted to the dentate gyrus and mossy fiber tract in mice heterozygous for α-calcium/calmodulin-dependent protein kinase II (Yamasaki *et al*, 2008). We have also shown that the dopaminergic modulation at the mossy fiber synapse is clearly enhanced in a subpopulation of mice lacking the schizophrenia susceptible gene dysbindin-1 (Kobayashi *et al*, 2011b). Our present results demonstrate that, in addition to these genetic factors, environmental factors can converge on this D_1-like receptor-mediated dopaminergic modulation to regulate mossy fiber synaptic transmission. The resultant changes in potentiation of synaptic transmission by dopamine might lead to alterations of hippocampus-dependent behaviors such as locomotor activity in novel environments (Kobayashi *et al*, 2006).

In contrast to marked upregulation of the D_1-like ligand binding, chronic fluoxetine did not cause any increase in the 5-HT_4 ligand binding in the hippocampus. Although the 5-HT_{1A} receptor binding was reduced in the CA1 region, there were no detectable changes in the dentate gyrus and

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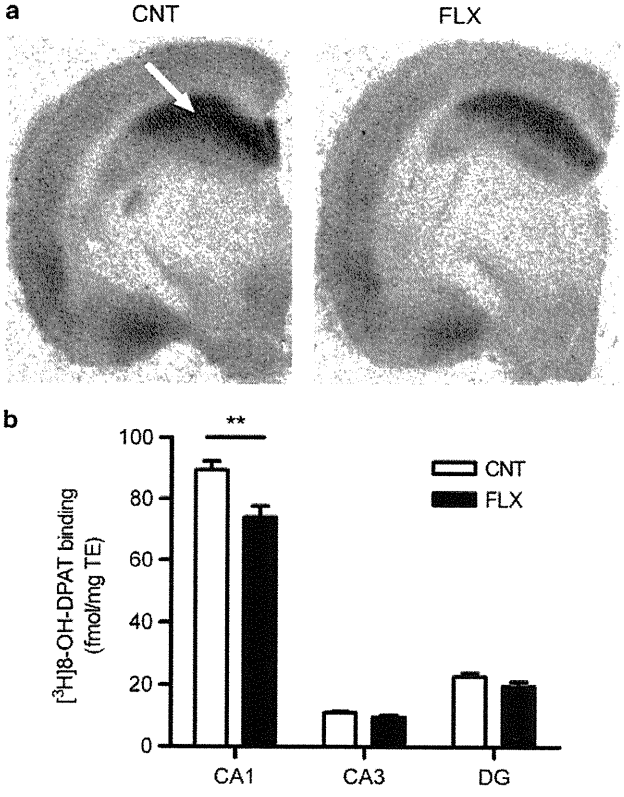

Figure 5 Reduced 5-HT_{1A} ligand binding in fluoxetine-treated mice (FLX). (a) Representative autoradiograms of [^{3}H]8-OHDPAT binding. The arrow indicates the hippocampal CA1 region. (b) Fluoxetine significantly reduced [^{3}H]8-OHDPAT binding in CA1 ($p = 0.007$) (control mice (CNT): $n = 6$, FLX: $n = 7$).

CA3 region. Therefore, the enhanced serotonin-induced potentiation at the mossy fiber synapse caused by fluoxetine cannot be ascribed to either upregulation of receptors mediating synaptic potentiation or downregulation of receptors mediating synaptic depression. Both 5-HT_4 and D_1-like receptors are coupled to Gs-adenylate cyclase pathways, and the monoaminergic modulation at the mossy fiber synapse depends on cAMP (Kobayashi and Suzuki, 2007; Kobayashi *et al*, 2008). Antidepressant drugs have been shown to facilitate interaction between Gs and adenylate cyclase (Donati and Rasenick, 2003). In some conditions, fluoxetine can reduce activity or expression of phosphodiesterase that metabolizes cAMP (Fatemi *et al*, 2010; Korff *et al*, 2009). Such altered downstream signaling may account for the enhanced 5-HT_4-dependent synaptic modulation in the FLX and may also contribute to the enhancement of the dopaminergic modulation at the mossy fiber synapse.

As the SSRI fluoxetine can inhibit dopamine reuptake at high concentrations (Sánchez and Hyttel, 1999), it is possible that the enhanced dopaminergic modulation could be caused by its direct action on the dopaminergic neurons. We showed that the effect of fluoxetine was abolished in mice treated with the serotonergic neurotoxin DHT. It has been shown that DHT injected into the brain region that is rich in dopaminergic terminals could change dopamine levels as well as serotonin at the injected region (Ludwig and Schwarting, 2007). However, intracerebroventricular injection of DHT as in the present study has been reported to generally spare dopaminergic neurons (Fischette *et al*, 1987; Reader and Gauthier, 1984; Winstanley *et al*, 2003). Therefore, the result of our DHT experiment suggests that the integrity of the serotonergic system is essential for the effect of fluoxetine on the dopaminergic modulation. In addition, whereas inhibition of dopamine reuptake generally causes immediate locomotor hyperactivity (Uhl *et al*, 2002), fluoxetine tends to reduce locomotor activity (Kobayashi *et al*, 2008, 2011a). Thus, although we cannot exclude the possibility of direct action of fluoxetine on the dopaminergic system, a contribution of such action, if any, to the effect of fluoxetine demonstrated in the present study is supposed to be small. Fluoxetine is known to antagonize the serotonin 5-HT_{2C} receptor (Sánchez and Hyttel, 1999). We showed that another SSRI PAR, which has a much lower affinity for 5-HT_{2C} (Sánchez and Hyttel, 1999), similarly augmented the dopaminergic synaptic modulation, confirming the importance of the serotonin reuptake inhibition rather the 5-HT_{2C} antagonism in the effect of fluoxetine. The mechanism that links the serotonin reuptake inhibition to the D_1-like receptor-dependent synaptic modulation is unknown. Brain-derived neurotrophic factor (BDNF) has been implicated in antidepressant action (Adachi *et al*, 2008; Malberg and Blendy, 2005). Chronic fluoxetine can increase both mRNA and mature protein levels of BDNF in the hippocampus (Musazzi *et al*, 2009), and BDNF has been shown to greatly increase the expression of D_1 receptor mRNA in the catecholaminergic CAD cell line (Do *et al*, 2007). Thus, it is possible that BDNF mediates the fluoxetine-induced increase in the D_1-like receptor expression levels in the hippocampus. Activation of cAMP response element-binding protein has been suggested to mediate an antidepressant-induced increase in BDNF transcription (Conti *et al*, 2002; Malberg and Blendy, 2005). Therefore, the upregulation of the 5-HT_4 receptor-dependent signaling coupled to cAMP elevation might be critically involved in the enhancement of D_1-like receptor-dependent synaptic modulation. However, in 5-HT_4-deficient mice, chronic fluoxetine significantly enhanced the dopaminergic modulation as in wild-type mice. The serotonin 5-HT_6 and 5-HT_7 receptors, which are also coupled to Gs-cAMP cascades, have been reported to be expressed in the dentate gyrus (Gérard *et al*, 1997; Neumaier *et al*, 2001; Vizuete *et al*, 1997). These receptors might have compensated for the lack of the 5-HT_4 receptor in the mutant mice. In the present study, we did not further examine the subtype of serotonin receptors mediating the effect of fluoxetine. Other subtypes of serotonin receptors are also expressed in the dentate gyrus (Klempin *et al*, 2010). The 5-HT_{1A} receptor mediates depression of mossy fiber synaptic transmission by serotonin (Kobayashi *et al*, 2008) and is involved in facilitation of the adult neurogenesis in the dentate gyrus by fluoxetine (Santarelli *et al*, 2003). The immunohistochemical analysis demonstrated abundant 5-HT_{2C}-like immunoreactivity in the granule cell layer (Klempin *et al*, 2010). These serotonin receptors might also have a role in mediating the effect of fluoxetine on the dopaminergic modulation at the mossy fiber synapse.

The dentate gyrus has been implicated in behavioral effects of SSRIs and other antidepressant drugs (Adachi

et al, 2008; Kobayashi *et al*, 2011a; Miyamoto *et al*, 2011; Santarelli *et al*, 2003). The selective upregulation of D_1-like ligand binding in the dentate gyrus-mossy fiber system by fluoxetine further supports the importance of the dentate gyrus in antidepressant action. Facilitated adult neurogenesis in the dentate gyrus has been suggested to be a candidate cellular process mediating antidepressant action (Malberg *et al*, 2000; Sahay and Hen, 2007; Santarelli *et al*, 2003). However, newly generated neurons constitute only a small fraction of dentate granule cells, and a recent study has shown that an increase in adult neurogenesis alone did not cause antidepressant-like behavioral effects (Sahay *et al*, 2011). We have previously shown that chronic SSRI treatments induce dematuration of dentate granule cells in adult mice (Kobayashi *et al*, 2010). The granule cell dematuration is induced in a large population of mature granule cells, significantly changes somatic excitability and synaptic plasticity at both input and output synapse of the granule cells, and is accompanied by marked enhancement of the serotonergic modulation at the mossy fiber synapse (Kobayashi *et al*, 2010). The present study has demonstrated that the potentiating effect of dopamine at the mossy fiber synapse is increased by about threefold after the fluoxetine treatment. These enhanced monoaminergic modulations would greatly increase excitatory drive to CA3 pyramidal cells by the mossy fibers. Furthermore, increased D_1-like receptor expression in the granule cells may facilitate the induction of long-term potentiation at the perforant path granule cell synapse (Hamilton *et al*, 2010; Kusuki *et al*, 1997). Together with changes in intrinsic functional properties of granule cells caused by dematuration, the altered monoaminergic synaptic modulation is likely to have a significant impact on propagation of neuronal signals through the dentate gyrus, thereby possibly contributing to neuronal bases for mechanisms of action of SSRIs.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

Adachi M, Barrot M, Autry AE, Theobald D, Monteggia LM (2008). Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. *Biol Psychiatry* **63**: 642–649.

Ainsworth K, Smith SE, Zetterström TSC, Pei Q, Franklin M, Sharp T (1998). Effect of antidepressant drugs on dopamine D_1 and D_2 receptor expression and dopamine release in the nucleus accumbens of the rat. *Psychopharmacology (Berl)* **140**: 470–477.

Chen CC, Yang CH, Huang CC, Hsu KS (2010). Acute stress impairs hippocampal mossy fiber-CA3 long-term potentiation by enhancing cAMP-specific phosphodiesterase 4 activity. *Neuropsychopharmacology* **35**: 1605–1617.

Conti AC, Cryan JF, Dalvi A, Lucki I, Blendy JA (2002). cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. *J Neurosci* **22**: 3262–3268.

D'Aquila PS, Collu M, Gessa GL, Serra G (2000). The role of dopamine in the mechanism of action of antidepressant drugs. *Eur J Pharmacol* **405**: 365–373.

D'Aquila PS, Collu M, Pani L, Gessa GL, Serra G (1994). Antidepressant-like effect of selective dopamine D_1 receptor agonists in the behavioural despair animal model of depression. *Eur J Pharmacol* **262**: 107–111.

Deslandes PN, Pache DM, Buckland P, Sewell RDE (2002). Morphine, cocaine and antidepressant induced motivational activity and midbrain dopaminergic neurotransmission. *Eur J Pharmacol* **453**: 223–229.

Do T, Kerr B, Kuzhikandathil EV (2007). Brain-derived neurotrophic factor regulates the expression of D_1 dopamine receptors. *J Neurochem* **100**: 416–428.

Donati RJ, Rasenick MM (2003). G protein signaling and the molecular basis of antidepressant action. *Life Sci* **73**: 1–17.

Dziedzicka-Wasylewska M, Willner P, Papp M (1997). Changes in dopamine receptor mRNA expression following chronic mild stress and chronic antidepressant treatment. *Behav Pharmacol* **8**: 607–618.

Fatemi SH, Folsom TD, Reutiman TJ, Braun NN, Lavergne LG (2010). Levels of phosphodiesterase 4A and 4B are altered by chronic treatment with psychotropic medications in rat frontal cortex. *Synapse* **64**: 550–555.

Fischette CT, Nock B, Renner K (1987). Effects of 5,7-dihydroxytryptamine on serotonin$_1$ and serotonin$_2$ receptors throughout the rat central nervous system using quantitative autoradiography. *Brain Res* **421**: 263–279.

Gambarana C, Ghiglieri O, Tagliamonte A, D'Alessandro N, de Montis MG (1995). Crucial role of D_1 dopamine receptors in mediating the antidepressant effect of imipramine. *Pharmacol Biochem Behav* **50**: 147–151.

Gérard C, Martres MP, Lefèvre K, Miquel MC, Vergé D, Lanfumey L *et al* (1997). Immuno-localization of serotonin 5-HT$_6$ receptor-like material in the rat central nervous system. *Brain Res* **746**: 207–219.

Hamilton TJ, Wheatley BM, Sinclair DB, Bachmann M, Larkum ME, Colmers WF (2010). Dopamine modulates synaptic plasticity in dendrites of rat and human dentate granule cells. *Proc Natl Acad Sci USA* **107**: 18185–18190.

Huzarska M, Zieliński M, Herman ZS (2006). Repeated treatment with antidepressants enhances dopamine D_1 receptor gene expression in the rat brain. *Eur J Pharmacol* **532**: 208–213.

Klempin F, Babu H, De Pietri Tonelli D, Alarcon E, Fabel K, Kempermann G (2010). Oppositional effects of serotonin receptors 5-HT1a, 2, and 2c in the regulation of adult hippocampal neurogenesis. *Front Mol Neurosci* **3**: 14.

Klimek V, Nielsen M (1987). Chronic treatment with antidepressants decreases the number of [^{3}H]SCH 23390 binding sites in the rat striatum and limbic system. *Eur J Pharmacol* **139**: 163–169.

Kobayashi K (2009). Targeting the hippocampal mossy fiber synapse for the treatment of psychiatric disorders. *Mol Neurobiol* **39**: 24–36.

Kobayashi K (2010). Hippocampal mossy fiber synaptic transmission and its modulation. *Vitam Horm* **82**: 65–85.

Kobayashi K, Ikeda Y, Haneda E, Suzuki H (2008). Chronic fluoxetine bidirectionally modulates potentiating effects of serotonin on the hippocampal mossy fiber synaptic transmission. *J Neurosci* **28**: 6272–6280.

Kobayashi K, Ikeda Y, Sakai A, Yamasaki N, Haneda E, Miyakawa T *et al* (2010). Reversal of hippocampal neuronal maturation by serotonergic antidepressants. *Proc Natl Acad Sci USA* **107**: 8434–8439.

Kobayashi K, Ikeda Y, Suzuki H (2006). Locomotor activity correlates with modifications of hippocampal mossy fibre synaptic transmission. *Eur J Neurosci* **24**: 1867–1873.

Kobayashi K, Ikeda Y, Suzuki H (2011a). Behavioral destabilization induced by the selective serotonin reuptake inhibitor fluoxetine. *Mol Brain* **4**: 12.

Kobayashi K, Poo M-M (2004). Spike train timing-dependent associative modification of hippocampal CA3 recurrent synapses by mossy fibers. *Neuron* **41**: 445–454.

Kobayashi K, Suzuki H (2007). Dopamine selectively potentiates hippocampal mossy fibre to CA3 synaptic transmission. *Neuropharmacology* **52**: 552–561.

Kobayashi K, Umeda-Yano S, Yamamori H, Takeda M, Suzuki H, Hashimoto R (2011b). Correlated alterations in serotonergic and dopaminergic modulations at the hippocampal mossy fiber synapse in mice lacking dysbindin. *PLoS One* **6**: e18113.

Korff S, Stein DJ, Harvey BH (2009). Cortico-striatal cyclic AMP-phosphodiesterase-4 signalling and stereotypy in the deer mouse: attenuation after chronic fluoxetine treatment. *Pharmacol Biochem Behav* **92**: 514–520.

Kusuki T, Imahori Y, Ueda S, Inokuchi K (1997). Dopaminergic modulation of LTP induction in the dentate gyrus of intact brain. *Neuroreport* **8**: 2037–2040.

Licht CL, Marcussen AB, Wegener G, Overstreet DH, Aznar S, Knudsen GM (2009). The brain 5-HT_4 receptor binding is down-regulated in the Flinders Sensitive Line depression model and in response to paroxetine administration. *J Neurochem* **109**: 1363–1374.

López-Giménez JF, Tecott LH, Palacios JM, Mengod G, Vilaró MT (2002). Serotonin 5-HT_{2C} receptor knockout mice: autoradiographic analysis of multiple serotonin receptors. *J Neurosci Res* **67**: 69–85.

Ludwig V, Schwarting RKW (2007). Neurochemical and behavioral consequences of striatal injection of 5,7-dihydroxytryptamine. *J Neurosci Methods* **162**: 108–118.

Magariños AM, Verdugo JMG, McEwen BS (1997). Chronic stress alters synaptic terminal structure in hippocampus. *Proc Natl Acad Sci USA* **94**: 14002–14008.

Malberg JE, Blendy JA (2005). Antidepressant action: to the nucleus and beyond. *Trends Pharmacol Sci* **26**: 631–638.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* **20**: 9104–9110.

Mansour A, Meador-Woodruff JH, Bunzow JR, Civelli O, Akil H, Watson SJ (1990). Localization of dopamine D_2 receptor mRNA and D_1 and D_2 receptor binding in the rat brain and pituitary: an *in situ* hybridization-receptor autoradiographic analysis. *J Neurosci* **10**: 2587–2600.

Meltzer HY, Massey BW (2011). The role of serotonin receptors in the action of atypical antipsychotic drugs. *Curr Opin Pharmacol* **11**: 59–67.

Miller BH, Schultz LE, Gulati A, Cameron MD, Pletcher MT (2008). Genetic regulation of behavioral and neuronal responses to fluoxetine. *Neuropsychopharmacology* **33**: 1312–1322.

Miyamoto D, Iijima M, Yamamoto H, Nomura H, Matsuki N (2011). Behavioural effects of antidepressants are dependent and independent on the integrity of the dentate gyrus. *Int J Neuropsychopharmacol* **14**: 967–976.

Musazzi L, Cattaneo A, Tardito D, Barbon A, Gennarelli M, Barlati S *et al* (2009). Early raise of BDNF in hippocampus suggests induction of posttranscriptional mechanisms by antidepressants. *BMC Neurosci* **10**: 48.

Neumaier JF, Sexton TJ, Yracheta J, Diaz AM, Brownfield M (2001). Localization of 5-HT_7 receptors in rat brain by immunocytochemistry, *in situ* hybridization, and agonist stimulated cFos expression. *J Chem Neuroanat* **21**: 63–73.

Reader TA, Gauthier P (1984). Catecholamines and serotonin in the rat central nervous system after 6-OHDA, 5-7-DHT and p-CPA. *J Neural Transm* **59**: 207–227.

Sahay A, Hen R (2007). Adult hippocampal neurogenesis in depression. *Nat Neurosci* **10**: 1110–1115.

Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS *et al* (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* **472**: 466–470.

Sampson D, Willner P, Muscat R (1991). Reversal of antidepressant action by dopamine antagonists in an animal model of depression. *Psychopharmacology (Berl)* **104**: 491–495.

Sánchez C, Hyttel J (1999). Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. *Cell Mol Neurobiol* **19**: 467–489.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S *et al* (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**: 805–809.

Suhara T, Nakayama K, Inoue O, Fukuda H, Shimizu M, Mori A *et al* (1992). D_1 dopamine receptor binding in mood disorders measured by positron emission tomography. *Psychopharmacology (Berl)* **106**: 14–18.

Uhl GR, Hall FS, Sora I (2002). Cocaine, reward, movement and monoamine transporters. *Mol Psychiatry* **7**: 21–26.

Vaswani M, Linda FK, Ramesh S (2003). Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. *Prog Neuropsychopharmacol Biol Psychiatry* **27**: 85–102.

Vergé D, Daval G, Marcinkiewicz M, Patey A, el Mestikawy S, Gozlan H *et al* (1986). Quantitative autoradiography of multiple 5-HT_1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. *J Neurosci* **6**: 3474–3482.

Vidal R, Valdizán EM, Mostany R, Pazos A, Castro E (2009). Long-term treatment with fluoxetine induces desensitization of 5-HT_4 receptor-dependent signalling and functionality in rat brain. *J Neurochem* **110**: 1120–1127.

Vizuete ML, Venero JL, Traiffort E, Vargas C, Machado A, Cano J (1997). Expression of 5-HT_7 receptor mRNA in rat brain during postnatal development. *Neurosci Lett* **227**: 53–56.

Winstanley CA, Dalley JW, Theobald DEH, Robbins TW (2003). Global 5-HT depletion attenuates the ability of amphetamine to decrease impulsive choice on a delay-discounting task in rats. *Psychopharmacology (Berl)* **170**: 320–331.

Yamasaki N, Maekawa M, Kobayashi K, Kajii Y, Maeda J, Soma M *et al* (2008). Alpha-CaMKII deficiency causes immature dentate gyrus, a novel candidate endophenotype of psychiatric disorders. *Mol Brain* **1**: 6.

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ORIGINAL INVESTIGATION

Striatal and extrastriatal dopamine D_2 receptor occupancy by the partial agonist antipsychotic drug aripiprazole in the human brain: a positron emission tomography study with [^{11}C]raclopride and [^{11}C]FLB457

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Abstract
Rationale Second-generation antipsychotics demonstrate clinical efficacy with fewer extrapyramidal side effects compared with first-generation antipsychotics. One of the proposed explanations is the hypothesis of preferential extrastriatal dopamine D_2 receptor occupancy (limbic selectivity) by antipsychotics. In the present study, we focused on aripiprazole, which has a unique pharmacological profile with partial agonism at dopamine D_2 receptors and the minimal risk of extrapyramidal side effects. Previous positron emission tomography (PET) studies using high-affinity radioligands for dopamine D_2 receptors have reported inconsistent results regarding regional differences of dopamine D_2 receptor occupancy by aripiprazole.

Objective To test the hypothesis of preferential binding to extrastriatal dopamine D_2 receptors by aripiprazole, we investigated its regional dopamine D_2 receptor occupancies in healthy young subjects.
Materials and methods Using PET and two radioligands with different affinities for dopamine D_2 receptors, [^{11}C]raclopride and [^{11}C]FLB457, striatal and extrastriatal dopamine D_2 receptor bindings at baseline and after oral administration of 6 mg aripiprazole were measured in 11 male healthy subjects.
Results Our data showed that dopamine D_2 receptor occupancies in the striatum measured with [^{11}C]raclopride were 70.1% and 74.1%, with the corresponding values for the extrastriatal regions measured with [^{11}C]FLB457 ranging from 46.6% to 58.4%.
Conclusions In the present study, preferential extrastriatal dopamine D_2 receptor occupancy by aripiprazole was not observed. Our data suggest partial agonism at dopamine D_2 receptors is the most likely explanation for the minimal risk of extrapyramidal side effects in the treatment by aripiprazole.

Keywords Antipsychotics · Dopamine D_2 receptor · Occupancy · Partial agonist · Aripiprazole

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Introduction

Since the first antipsychotic drug appeared in the mid-twentieth century with the introduction of chlorpromazine, antipsychotic drugs have been the first-line treatment for schizophrenia and related psychotic disorders. Molecular

imaging studies have explored potential pathways to the manifestation of clinical efficacy of various antipsychotics. Past studies revealed that dopamine D_2 receptor occupancy measurements provide a valid predictor of antipsychotic responses and extrapyramidal side effects (Farde et al. 1992; Kapur et al. 2000; Kapur et al. 1999; Nordström et al. 1993).

Second-generation, or atypical, antipsychotics are effective in the treatment of both positive and negative symptoms of schizophrenia. Compared to first-generation antipsychotics, they cause significantly fewer and less severe extrapyramidal side effects and prolactin level elevations (Leucht et al. 2009). Several molecular imaging studies using high-affinity radioligands for dopamine D_2 receptors have reported regional differences of dopamine D_2 receptor occupancy by second-generation antipsychotics. Initially, using [^{123}I]epidepride, Pilowsky et al. (1997) reported "limbic selectivity" of clozapine, i.e., higher dopamine D_2 receptor occupancies in the extrastriatal regions than in the striatum. Similar findings were obtained with clozapine by PET studies using [^{76}Br]FLB457 (Xiberas et al. 2001) and [^{18}F]fallypride (Gründer et al. 2006; Kessler et al. 2006). In contrast, first-generation antipsychotics were reported to show similar dopamine D_2 receptor occupancies in striatal and extrastriatal regions, as measured with [^{123}I]epidepride (Bigliani et al. 1999; Pilowsky et al. 1997). Based on these findings, the hypothesis of the preferential extrastriatal dopamine D_2 receptor occupancy has been suggested to explain the actions of second-generation antipsychotics (Pilowsky et al. 1997). Preferential extrastriatal dopamine D_2 receptor occupancy was also shown in other second-generation antipsychotics, such as risperidone using [^{76}Br]FLB457 (Xiberas et al. 2001) and [^{123}I]epidepride (Bressan et al. 2003), olanzapine using [^{76}Br]FLB457 (Xiberas et al. 2001), and quetiapine using [^{18}F]fallypride (Kessler et al. 2006) in patients with schizophrenia. However, the results of several other molecular imaging studies were inconsistent. For example, studies combining [^{11}C]FLB457 imaging for extrastriatal D_2 receptors and [^{11}C]raclopride imaging for striatal D_2 receptors have reported no differences in occupancy of dopamine D_2 receptors between the striatal and extrastriatal regions in schizophrenia patients taking clozapine (Talvik et al. 2001). The absence of preferential binding to extrastriatal dopamine D_2 receptors was also reported with risperidone (Ito et al. 2009), olanzapine (Arakawa et al. 2010; Kessler et al. 2005), and paliperidone (Arakawa et al. 2007).

In the present study, we focused on aripiprazole, which differs from the above-mentioned first- and second-generation antipsychotics in its unique pharmacological profile. Aripiprazole acts as a partial agonist at dopamine D_2 and serotonin 5-HT_{1A} receptors and as an antagonist at serotonin 5-HT_2 receptors (Burris et al. 2002; Davies et al. 2006). It has been shown to be as effective as haloperidol and risperidone in the treatment of positive and negative symptoms of schizophrenia and schizoaffective disorder. Furthermore, a lower incidence of the known side effects associated with antipsychotics such as extrapyramidal symptoms, weight gain, tardive dyskinesia, prolactin level elevation, and sedation has been observed (Bhattacharjee and El-Sayeh 2008). A PET study using [^{11}C] raclopride reported striatal occupancy values ranging from 60% to 95% without notable extrapyramidal side effects (Yokoi et al. 2002). Preferential extrastriatal dopamine D_2 receptor occupancy by aripiprazole has also been tested in two PET studies using [^{18}F]fallypride in patients diagnosed with schizophrenia (Gründer et al. 2008; Kegeles et al. 2008). Kegeles et al. (2008) reported higher dopamine D_2 receptor occupancies in the extrastriatum compared to the striatum in patients with schizophrenia and schizoaffective disorder treated with aripiprazole. However, in another PET study using the same radioligand, Gründer et al. (2008) reported no regional difference in dopamine D_2 receptor occupancy across brain regions by aripiprazole in 16 patients with schizophrenia or schizoaffective disorder.

The purpose of the present study was to test the hypothesis of preferential binding to extrastriatal dopamine D_2 receptors by aripiprazole. Striatal and extrastriatal dopamine D_2 receptor bindings at baseline and after oral administration of 6 mg aripiprazole were measured using PET in the same healthy subjects. Based on the finding that D_2 receptor densities are quite different between the striatal and extrastriatal regions (Hall et al. 1996; Hall et al. 1994), dopamine D_2 receptor bindings were measured using two radioligands with different affinities—[^{11}C]raclopride for the striatum and [^{11}C]FLB457 for the extrastriatal regions (Farde et al. 1995; Suhara et al. 1999).

Materials and methods

Subjects

The study was approved by the Institutional Review Board of the National Institute of Radiological Sciences, Chiba, Japan. Eleven healthy male subjects [20–35 years, 23.7±4.0 (mean ± S.D.)] participated in the study. Written informed consent was obtained from each subject after complete explanation of the study. Subjects with current or past psychiatric disorders, substance abuse, or organic brain disease were excluded on the basis of their medical history and magnetic resonance (MR) imaging of the brain. Subjects also underwent a physical examination and blood and urine analysis to exclude physical illness.

PET procedures

All PET studies were performed with a Siemens ECAT HR + system, which provides 63 sections with an axial field of view of 15.5 cm (Brix et al. 1997). The intrinsic spatial resolution