($p < 0.05$, FWE-corrected) (Table 2, Fig. 1A). In contrast, there were no areas with significantly greater regional GMVs in the patients with MAP compared with the HC subjects. With regard to WMV, VBM identified an orbitofrontal area (OFA) with a significantly smaller WMV in the patients with MAP compared with the HC subjects ($p < 0.05$, FWE-corrected). There were no regions with significantly larger WMVs in the individuals with MAP compared with the HC subjects (Fig. 1B). The statistical conclusions for differences in regional brain volumes were preserved when the group difference in the estimated IQ, in which the patients showed significantly low level compared with the HC subjects, was accounted by employing this variable as a nuisance covariate in the group comparisons.

3.3. Correlates of gray or white matter with clinical variables in patients with MAP

A significant correlation between smaller regional GMVs in the anterior rostral part of the medial portion of the FPC and higher BPRS positive symptom scores was found in the patients with MAP ([−14 50 1], $Z = 3.28$, $p < 0.001$). There was no significant correlation between regional GMVs or WMVs and the clinical information or potential confounds.

4. Discussion

To our knowledge, the present study is the first VBM study to demonstrate regional brain volume reductions in patients with MAP. The main findings are significant GMV reductions in the left perisylvian regions, including the posterior IFG and anterior STG, and approximately in the frontopolar Brodmann area (BA) 10, including its dorsomedial, ventromedial, dorsolateral, and ventrolateral portions, and WMV reductions in the OFA in the patients with MAP compared with the HC subjects. In addition, correlation analysis demonstrated a significant correlation between smaller regional GMVs in the anterior rostral part of medial portion of the FPC and higher BPRS positive symptom scores in the patients with MAP.

Previous studies have consistently reported volume reductions in left perisylvian structures, such as the STG and the IFG, as described in meta-analyses of VBM studies involving both first episode psychosis (Chan et al., 2011; Fusar-Poli et al., 2012) and chronic schizophrenia (Bora et al., 2011; Chan et al., 2011). The importance of the IFG in the pathophysiology of schizophrenia was also supported by our previous volumetric studies focusing on the IFG that suggested that localized GMV reductions of the pars triangularis and pars opecularis represent biomarkers for vulnerability to and those for the clinical severity and the progression of schizophrenia, respectively (Suga et al., 2010; Iwashiro et al., 2012). With regard to the STG, neuroimaging studies identified portions of the bilateral anterior STG (including part of Heschl's gyri) as key areas in the structural pathophysiology of auditory verbal hallucinations (Kasai et al., 2003; Yamasue et al., 2004; Yoshida et al., 2009; Takahashi et al., 2010; Molina et al., 2011; Asami et al., 2012; Modinos et al., 2013). It has also been suggested that volume reductions in the STG, especially the left hemisphere (Hirayasu et al., 1998) are specific to schizophrenia rather than affective psychosis (McCarley et al., 2002). Based on these previous studies, GMV reductions in the left perisylvian structures, such as the IFG and the STG,

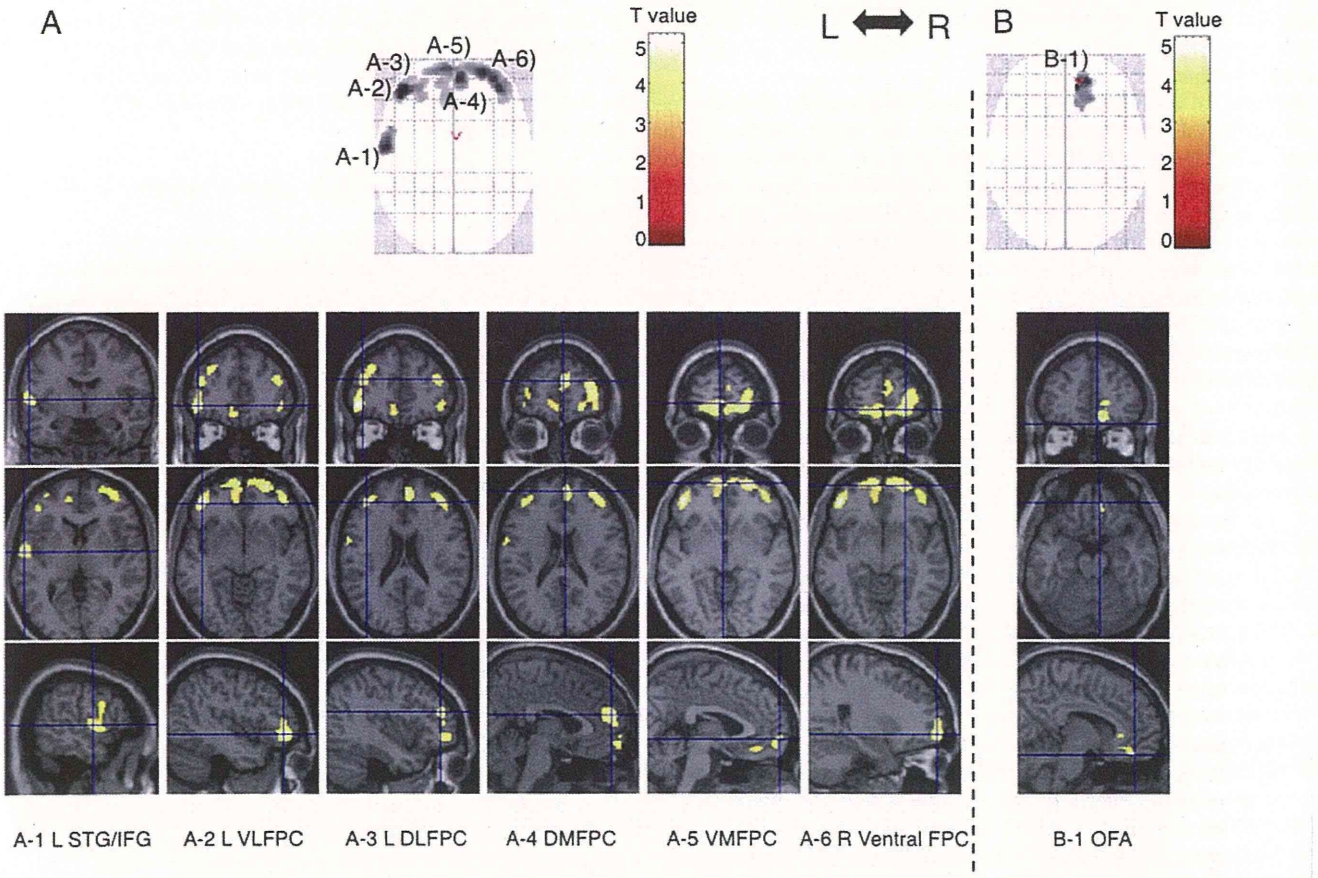

Fig. 1. Regions of brain volume reductions in patients with methamphetamine-induced psychosis. A 1-6) Areas with significantly reduced gray matter volumes in the patients with methamphetamine-induced psychosis compared with the healthy control subjects rendered onto orthogonal slices (voxel threshold $p < 0.001$). STG: superior temporal gyrus; IFG: inferior frontal gyrus; VLFPC: ventrolateral frontopolar cortex; DLFPC: dorsolateral frontopolar cortex; DMFPC: dorsomedial frontopolar cortex; VMFPC: ventromedial frontopolar cortex. B) Areas with significantly reduced white matter volumes in the patients with methamphetamine-induced psychosis compared with the healthy controls rendered onto orthogonal slices (voxel threshold $p < 0.001$). OFA: orbitofrontal area.

in patients with MAP may be a crucial factor in the development of schizophrenia-like psychotic symptoms such as auditory hallucinations. Thus, GMV reductions in these left perisylvian structures can be considered to be a common neurobiological underpinning for MAP and schizophrenia.

The current study identified extensive GMV reductions approximately in the frontopolar BA10 cortices, including in its dorsal to ventral and medial to lateral portions, in the patients with MAP. A great majority of previous meta-analyses of volumetric studies did not demonstrate GMV reduction in the BA 10 in individuals with schizophrenia (Glahn et al., 2008) or first episode psychosis (Fusar-Poli et al., 2012). In particular, although medial portions of the FPC have been reported to be decreased in patients with schizophrenia, gray matter deficits in the dorsal portions of the FPC have rarely been reported (Glahn et al., 2008; Fusar-Poli et al., 2012). On the other hand, previous VBM studies of people with METH dependence have repeatedly shown decreased GMVs in the BA 10 (Kim et al., 2006; Morales et al., 2012). Thus, the extent of volume reductions in BA 10 is considered to be a relatively specific finding to METH-related psychiatric problems.

The polar sector in the BA 10 modulates behavior by regulating emotional reactivity to perceived environmental threats or frustration in the absence of an expected reward, and instrumental violence is hypothesized to be associated with abnormalities within the anterior medial FPC (Anderson et al., 1999). Notably, a number of studies have reported that the anterior rostral part of the medial portion of the BA 10 is a neural basis of moral judgment (e.g. refs. Greene et al., 2001; Moll et al., 2006). These findings suggest that dysfunction in an area from the anterior rostral part of the medial portion of the FPC may result in antisocial and immoral behavior. As these consequences may increase BPRS scores, it is important to note that severe positive symptoms were correlated with smaller GMVs in the anterior rostral part of the medial portion of FPC in the current participants with MAP.

On the other hand, the current VBM also identified WMV reduction in the OFA. The area has been recognized to involve in appetitive behavior and decision making, in particular with regard to expectations of outcome, whose disturbance is associated with several types of antisocial behavior, such as substance abuse and dependence (Lucantonio et al., 2012), with comorbid dysfunction of amygdala area (Koob, 1996; Chase et al., 2011). Thus, volume reduction in the OFA potentially reflects neural basis of antisocial behavior, such as inappropriate usage of METH in the current participants.

The absence of a group of patients with schizophrenia is a limitation of the current study. Although we have cited results from previous studies that investigated volumetric changes in patients with schizophrenia to indirectly compare those patients with the patients in the current study, this indirect comparison ignores the possible confounds of various technological differences between studies. These confounds can be addressed with a direct comparison. Highly T1-weighted images likely underestimate the cortical volumes because they are influenced by infiltration of myelin into the gray matter (Bartzokis et al., 2009; Glasser and Van Essen, 2011). Thus, although our analyses revealed areas of GMV reductions in patients with MAP compared with healthy control subjects, it is not possible using this design to determine areas of GMV loss that are specific to patients with MAP.

In addition, to establish the clinical characteristics of MAP that distinguish it from schizophrenia, the clinical features of patients with MAP that are distinct from those of patients with schizophrenia should be noted carefully. At first, none of the patients with MAP experienced psychotic symptoms before their first consumption of METH. However, their repeated consumption of METH eventually resulted in psychotic symptoms, but concordant with the ICD-10 criteria of MAP, each episode of psychosis remitted within 6 months. In accord with traditional description of MAP (Jönsson et al., 1971; Rylander, 1972; Anggard et al., 1973), in six out of the twenty patients, psychotic symptoms disappeared within a week. After the first episode of MAP, the patients were prescribed antipsychotics to prevent subsequent psychotic episodes.

Here we discuss the methodological considerations and limitations of the present study. First, although the sample size was sufficient to reveal volumetric difference between the patients and controls, the number of patients was insufficient to detect potential differences within patient subtypes, such as patients with transient versus prolonged psychosis, users with a history of intravenous injection versus smoking METH, and male versus female patients. Second, although whether neuroleptics affect brain morphology remains controversial and the current VBM analysis identified no relationship between antipsychotic dosing and regional brain volumes, use of neuroleptics in the patients with MAP is a potential limitation. Some previous studies have reported regional brain volume reductions related to the cumulative neuroleptic dose to which an individual has been exposed (Nopoulos et al., 2001; Heitmiller et al., 2004), while others reported no significant changes or increases in regional brain volumes (Lieberman et al., 2005: Girgis et al., 2006). These inconsistent results may possibly stem from differences between the participants and/or neuroleptics examined. Based on these previous studies, it has been accepted that neuroleptics, particularly typicals rather than atypicals, affect the basal ganglia and thalamus, but it remains controversial whether neuroleptics affect cortical volumes (reviewed in Navari and Dazzan, 2009). Thus, although the correlation analyses demonstrated no significant correlation between GMV alterations and the durations of consumption of psychotropic medications and chlorpromazine equivalents, GMV alteration can be influenced by either neuroleptics or METH (Bartzokis., 2012). In addition, GMV changes identified by VBM are an indirect reflection of underlying biological changes, rather than direct observations of anatomy. Thus, the current findings should be interpreted with caution (Bartzokis et al., 2009). Third, although the VBM approach used in this study revealed significant volume reductions in the frontal and temporal lobe, the cross-sectional design of the current study precluded us from determining whether the current findings represent a predisposition to or consequence of the subject's neuropsychiatric status. Fourth, it is a reasonable criticism that we did not compare volumetric changes between METH-dependent patients who did or did not experience MAP, as it is unclear whether the volumetric changes identified by the current VBM approach are related to MAP or exposure to methamphetamine. It has been reported that 75% of METH users experience at least mild suspiciousness and unusual thoughts or hallucinations each year. Furthermore, 27% of patients with METH dependence develop clinically significant psychosis each year (McKetin et al., 2006). Thus, it is difficult to recruit sufficient METH-dependent individuals who have not experienced psychotic symptoms to form an appropriate control group.

In conclusion, the present VBM study identified that patients with MAP showed regional GMV reductions that are similar to those that have been reported in patients with schizophrenia. These GMV reductions were localized in the left perisylvian region and may be a common neural basis for similar psychotic symptoms. On the other hand, the VBM also detected BA 10 dominant GMV reductions that are not commonly observed in individuals with schizophrenia and may be associated with symptoms of MAP. However, as we did not conduct a direct comparison between patients with MAP and schizophrenia, there remains the possibility that the differences between the present study and previous studies derive from technical differences rather than differences between MAP and schizophrenia. We expect that future research will overcome this limitation of the current study.

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Contributors

Y.A., K.K., and H.Y. contributed to project management. Y.A. and H.Y. wrote the manuscript. L.O., and Y.T. made clinical evaluations. L.O., Y.T., Y.M., Y.S., and Y.O., made effort for the recruitment of the participants. Y.S. and N.Y. advised and supported MR imaging processing. T.I. supervised MR imaging acquisitions and evaluated all of the acquired images. All authors including M.I., M.S., and M.K. contributed to the critical revision and final approval of the manuscript.

Conflict of interest

All of the authors reported no biomedical financial interests or potential conflicts of interest.

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References

Anderson, S.W., Bechara, A., Damasio, H., Tranel, D., Damasio, A.R., 1999. Impairment of social and moral behavior related to early damage in human prefrontal cortex. Nat. Neurosci. 2, 1032–1037.

Anggard, E., Jonsson, L.E., Hogmark, A.L., Eng, C., Gunne, L.M., 1973. Amphetamine metabolism in amphetamine psychosis. Clin. Pharmacol. Ther. 14, 870–880.

Asami, T., Bouix, S., Whitford, T.J., Shenton, M.E., Salisbury, D.F., McCarley, R.W., 2012. Longitudinal loss of gray matter volume in patients with first-episode schizophrenia: DARTEL automated analysis and ROI validation. NeuroImage 59, 986–996.

Ashburner, J., 2007. A fast diffeomorphic image registration algorithm. NeuroImage 38, 95–113.

Ashburner, J., Friston, K.J., 2000. Voxel-based morphometry—the methods. NeuroImage 11, 805–821.

Bartzokis, G., 2012. Neuroglialpharmacology: myelination as a shared mechanism of action of psychotropic treatments. Neuropharmacology 62, 2137–2153.

Bartzokis, G., Lu, P.H., Stewart, S.B., Oluwadara, B., Lucas, A.J., Pantages, J., Pratt, E., Sherin, J.E., Altshuler, L.L., Mintz, J., Gitlin, M.J., Subotnik, K.L., Nuechterlein, K.H., 2009. In vivo evidence of differential impact of typical and atypical antipsychotics on intracortical myelin in adults with schizophrenia. Schizophr. Res. 113, 322–331.

Bora, E., Fornito, A., Radua, J., Walterfang, M., Seal, M., Wood, S.J., Yücel, M., Velakoulis, D., Pantelis, C., 2011. Neuroanatomical abnormalities in schizophrenia: a multimodal voxelwise meta-analysis and meta-regression analysis. Schizophr. Res. 127, 46–57.

Callaghan, R.C., Cunningham, J.K., Allebeck, P., Arenovich, T., Sajeev, G., Remington, G., Boileau, I., Kish, S.J., 2012. Methamphetamine use and schizophrenia: a population-based cohort study in California. Am. J. Psychiatry 169, 389–396.

Chan, R.C.K., Di, X., McAlonan, G.M., Gong, Q.-Y., 2011. Brain anatomical abnormalities in high-risk individuals, first-episode, and chronic schizophrenia: an activation likelihood estimation meta-analysis of illness progression. Schizophr. Bull. 37, 177–188.

Chase, H.W., Eickhoff, S.B., Laird, A.R., Hogarth, L., 2011. The neural basis of drug stimulus processing and craving: an activation likelihood estimation meta-analysis. Biol. Psychiatry 70, 785–793.

Ellinwood, E.H., 1968. Amphetamine psychosis: II. Theoretical implication. Int. J. Neuropsychiatry 4, 45–54.

Ellinwood, E.H., Sudilovsky, A., Nelson, L.M., 1973. Evolving behavior in the clinical and experimental amphetamine model psychosis. Am. J. Psychiatry 130, 1088–1093.

Flaum, M., Schultz, S.K., 1996. When does amphetamine-induced psychosis become schizophrenia? Am. J. Psychiatry 153, 812–815.

Fusar-Poli, P., Radua, J., McGuire, P., Borgwardt, S., 2012. Neuroanatomical maps of psychosis onset: voxel-wise meta-analysis of antipsychotic-naive VBM studies. Schizophr. Bull. 38, 1297–1307.

Girgis, R.R., Diwadkar, V.A., Nutche, J.J., Sweeney, J.A., Keshavan, M.S., Hardan, A.Y., 2006. Risperidone in first-episode psychosis: a longitudinal, exploratory voxel-based morphometric study. Schizophr. Res. 82, 89–94.

Glahn, D.C., Laird, A.R., Ellison-Wright, I., Thelen, S.M., Robinson, J.L., Lancaster, J.L., Bullmore, E., Fox, P.T., 2008. Meta-analysis of gray matter anomalies in schizophrenia: application of anatomic likelihood estimation and network analysis. Biol. Psychiatry 64, 774–781.

Glasser, M.F., Van Essen, D.C., 2011. Mapping human cortical areas in vivo based on myelin content as revealed by t1- and t2-weighted MRI. J. Neurosci. 31, 1597–1616.

Grant, K.M., LeVan, T.D., Wells, S.M., Li, M., Stoltenberg, S.F., Gendelman, H.E., Carlo, G., Bevins, R.A., 2012. Methamphetamine-associated psychosis. J. Neuroimmune Pharmacol. 7, 113–139.

Greene, J.D., Sommerville, R.B., Nystrom, L.E., Darley, J.M., Cohen, J.D., 2001. An fMRI investigation of emotional engagement in moral judgment. Science 293, 2105–2108.

Grelotti, D.J., Kanayama, G., Pope, H.G., 2010. Remission of persistent methamphetamine-induced psychosis after electroconvulsive therapy: presentation of a case and review of the literature. Am. J. Psychiatry 167, 17–23.

Heitmiller, D.R., Nopoulos, P.C., Andreasen, N.C., 2004. Changes in caudate volume after exposure to atypical neuroleptics in patients with schizophrenia may be sex-dependent. Schizophr. Res. 66, 137–142.

Hirayasu, Y., Shenton, M.E., Salisbury, D.F., Dickey, C.C., Fischer, I.A., Mazzoni, P., Kisler, T., Arakaki, H., Kwon, J.S., Anderson, J.E., Yurgelun-Todd, D., Tohen, M., McCarley, R.W., 1998. Lower left temporal lobe MRI volumes in patients with first-episode schizophrenia compared with psychotic patients with first-episode affective disorder and normal subjects. Am. J. Psychiatry 155, 1384–1391.

Hollingshead, A.d.B., 1957. Two Factor Index of Social Position. Yale University, Dept. of Sociology, New Haven, Conn.

Iwanami, A., Kato, N., Nakatani, Y., 1991. P300 in methamphetamine psychosis. Biol. Psychiatry 30, 726–730.

Iwashiro, N., Suga, M., Takano, Y., Inoue, H., Natsubori, T., Satomura, Y., Koike, S., Yahata, N., Murakami, M., Katsura, M., Gonoi, W., Sasaki, H., Takao, H., Abe, O., Kasai, K., Yamasue, H., 2012. Localized gray matter volume reductions in the pars triangularis of the inferior frontal gyrus in individuals at clinical high-risk for psychosis and first episode for schizophrenia. Schizophr. Res. 137, 124–131.

Jönsson, L.E., Lewander, T., Gunne, L.M., 1971. Amphetamine psychosis: urinary excretion of catecholamines and concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid. Res. Commun. Chem. Pathol. Pharmacol. 2, 355–369.

Kasai, K., Shenton, M.E., Salisbury, D.F., Hirayasu, Y., Onitsuka, T., Spencer, M.H., Yurgelun-Todd, D.A., Kikinis, R., Jolesz, F.A., McCarley, R.W., 2003. Progressive decrease of left Heschl gyrus and planum temporale gray matter volume in first-episode schizophrenia: a longitudinal magnetic resonance imaging study. Arch. Gen. Psychiatry 60, 766–775.

Kim, S.J., Lyoo, I.K., Hwang, J., Chung, A., Hoon Sung, Y., Kim, J., Kwon, D.-H., Chang, K.H., Renshaw, P.F., 2006. Prefrontal grey-matter changes in short-term and long-term abstinent methamphetamine abusers. Int. J. Neuropsychopharmacol. 9, 221–228.

Kishimoto, M., Ujike, H., Motohashi, Y., Tanaka, Y., Okahisa, Y., Kotaka, T., Harano, M., Inada, T., Yamada, M., Komiyama, T., Hori, T., Sekine, Y., Iwata, N., Sora, I., Iyo, M., Ozaki, N., Kuroda, S., 2008. The dysbindin gene (DTNBP1) is associated with methamphetamine psychosis. Biol. Psychiatry 63, 191–196.

Koob, G.F., 1996. Drug addiction: the yin and yang of hedonic homeostasis. Neuron 16, 893–896.

Laakso, M.P., Gunning-Dixon, F., Vaurio, O., Repo-Tiihonen, E., Soininen, H., Tiihonen, J., 2002. Prefrontal volumes in habitually violent subjects with antisocial personality disorder and type 2 alcoholism. Psychiatry Res. 114, 95–102.

Lieberman, J.A., Tollefson, G.D., Charles, C., Zipursky, R., Sharma, T., Kahn, R.S., Keefe, R.S., Green, A.I., Gur, R.E., McEvoy, J., Perkins, D., Hamer, R.M., Gu, H., Tohen, M., HGDH Study Group, 2005. Antipsychotic drug effects on brain morphology in first-episode psychosis. Arch. Gen. Psychiatry 62, 61–70.

Lucantonio, F., Stalnaker, T.A., Shaham, Y., Niv, Y., Schoenbaum, G., 2012. The impact of orbitofrontal dysfunction on cocaine addiction. Nat. Neurosci. 15, 358–366.

Maldjian, J.A., Laurienti, P.J., Kraft, R.A., Burdette, J.H., 2003. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. NeuroImage 19, 1233–1239.

Matsuoka, K., Uno, M., Kasai, K., Koyama, K., Kim, Y., 2006. Estimation of premorbid IQ in individuals with Alzheimer's disease using Japanese ideographic script (Kanji) compound words: Japanese version of National Adult Reading Test. Psychiatry Clin. Neurosci. 60, 332–339.

Matsuzawa, D., Hashimoto, K., Miyatake, R., Shirayama, Y., Shimizu, E., Maeda, K., Suzuki, Y., Mashimo, Y., Sekine, Y., Inada, T., Ozaki, N., Iwata, N., Harano, M., Komiyama, T., Yamada, M., Sora, I., Ujike, H., Hata, A., Sawa, A., Iyo, M., 2007. Identification of functional polymorphisms in the promoter region of the human PICK1 gene and their association with methamphetamine psychosis. Am. J. Psychiatry 164, 1105–1114.

McCarley, R.W., Salisbury, D.F., Hirayasu, Y., Yurgelun-Todd, D.A., Tohen, M., Zarate, C., Kikinis, R., Jolesz, F.A., Shenton, M.E., 2002. Association between smaller left posterior superior temporal gyrus volume on magnetic resonance imaging and smaller left temporal P300 amplitude in first-episode schizophrenia. Arch. Gen. Psychiatry 59, 321–331.

McKetin, R., McLaren, J., Lubman, D.I., Hides, L., 2006. The prevalence of psychotic symptoms among methamphetamine users. Addiction 101, 1473–1478.

Modinos, G., Costafreda, S.G., van Tol, M.-J., McGuire, P.K., Aleman, A., Allen, P., 2013. Neuroanatomy of auditory verbal hallucinations in schizophrenia: a quantitative meta-analysis of voxel-based morphometry studies. Cortex 49, 1046–1055.

Molina, V., Galindo, G., Cortés, B., de Herrera, A.G.S., Ledo, A., Sanz, J., Montes, C., Hernández-Tamames, J.A., 2011. Different gray matter patterns in chronic schizophrenia and chronic bipolar disorder patients identified using voxel-based morphometry. Eur. Arch. Psychiatry Clin. Neurosci. 261, 313–322.

Moll, J., Krueger, F., Zahn, R., Pardini, M., de Oliveira-Souza, R., Grafman, J., 2006. Human fronto-mesolimbic networks guide decisions about charitable donation. Proc. Natl. Acad. Sci. U. S. A. 103, 15623–15628.

Morales, A.M., Lee, B., Hellemann, G., O'Neill, J., London, E.D., 2012. Gray-matter volume in methamphetamine dependence: cigarette smoking and changes with abstinence from methamphetamine. Drug Alcohol Depend. 125, 230–238.

Müller, J.L., Gänßbauer, S., Sommer, M., Döhnel, K., Weber, T., Schmidt-Wilcke, T., Hajak, G., 2008. Gray matter changes in right superior temporal gyrus in criminal psychopaths. Evidence from voxel-based morphometry. Psychiatry Res. 163, 213–222.

Navari, S., Dazzan, P., 2009. Do antipsychotic drugs affect brain structure? A systematic and critical review of MRI findings. Psychol. Med. 39, 1763–1777.

Nelson, H.E., 1982. National Adult Reading Test (NART). NFER-Nelson, Windsor, UK.

Nopoulos, P.C., Ceilley, J.W., Gailis, E.A., Andreasen, N.C., 2001. An MRI study of midbrain morphology in patients with schizophrenia: relationship to psychosis, neuroleptics, and cerebellar neural circuitry. Biol. Psychiatry 49, 13–19.

Orikabe, L., Yamasue, H., Inoue, H., Takayanagi, Y., Mozue, Y., Sudo, Y., Ishii, T., Itokawa, M., Suzuki, M., Kurachi, M., Okazaki, Y., Kasai, K., 2011. Reduced amygdala and hippocampal volumes in patients with methamphetamine psychosis. Schizophr. Res. 132, 183–189.

Overall, J.E., Donald, R.G., 1962. The brief psychiatric rating scale. Psychol. Rep. 10, 799–812.
Pantelis, C., Velakoulis, D., McGorry, P.D., Wood, S.J., Suckling, J., Phillips, L.J., Yung, A.R., Bullmore, E.T., Brewer, W., Soulsby, B., Desmond, P., McGuire, P.K., 2003. Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. Lancet 361, 281–288.
Raine, A., Lencz, T., Bihrle, S., LaCasse, L., Colletti, P., 2000. Reduced prefrontal gray matter volume and reduced autonomic activity in antisocial personality disorder. Arch. Gen. Psychiatry 57, 119–128.
Rounsaville, B.J., 2007. DSM-V research agenda: substance abuse/psychosis comorbidity. Schizophr. Bull. 33, 947–952.
Rylander, G., 1972. Psychoses and the pudding and choreiform syndromes in addiction to central stimulant drug. Psychiatr. Neurol. Neurochir. 75, 203–212.
Sato, M., Numachi, Y., Hamamura, T., 1992. Relapse of paranoid psychotic state in methamphetamine model of schizophrenia. Schizophr. Bull. 18, 115–122.
Sekine, Y., Iyo, M., Ouchi, Y., Matsunaga, T., Tsukada, H., Okada, H., Yoshikawa, E., Futatsubashi, M., Takei, N., Mori, N., 2001. Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. Am. J. Psychiatry 158, 1206–1214.
Sekine, Y., Minabe, Y., Ouchi, Y., Takei, N., Iyo, M., Nakamura, K., Suzuki, K., Tsukada, H., Okada, H., Yoshikawa, E., Futatsubashi, M., Mori, N., 2003. Association of dopamine transporter loss in the orbitofrontal and dorsolateral prefrontal cortices with methamphetamine-related psychiatric symptoms. Am. J. Psychiatry 160, 1699–1701.
Sled, J.G., Zijdenbos, A.P., Evans, A.C., 1998. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. IEEE Trans. Med. Imaging 17, 87–97.
Substance Abuse and Mental Health Services Administration office of applied studies, 2012. Results from the 2007 National Survey on Drug Use and Health.
Suga, M., Yamasue, H., Abe, O., Yamasaki, S., Yamada, H., Inoue, H., Takei, K., Aoki, S., Kasai, K., 2010. Reduced gray matter volume of Brodmann's Area 45 is associated with severe psychotic symptoms in patients with schizophrenia. Eur. Arch. Psychiatry Clin. Neurosci. 260, 465–473.
Takahashi, T., Suzuki, M., Zhou, S.-Y., Tanino, R., Nakamura, K., Kawasaki, Y., Seto, H., Kurachi, M., 2010. A follow-up MRI study of the superior temporal subregions in schizotypal disorder and first-episode schizophrenia. Schizophr. Res. 119, 65–74.
Takayanagi, Y., Kawasaki, Y., Nakamura, K., Takahashi, T., Orikabe, L., Toyoda, E., Mozue, Y., Sato, Y., Itokawa, M., Yamasue, H., Kasai, K., Kurachi, M., Okazaki, Y., Matsushita, M., Suzuki, M., 2010. Differentiation of first-episode schizophrenia patients from healthy controls using ROI-based multiple structural brain variables. Prog. Neuropsychopharmacol. Biol. Psychiatry 34, 10–17.
Tsujimoto, S., Genovesio, A., Wise, S.P., 2011. Frontal pole cortex: encoding ends at the end of the endbrain. Trends Cogn. Sci. 15, 169–176.
Verhoeff, N.P., Meyer, J.H., Kecojevic, A., Hussey, D., Lewis, R., Tauscher, J., Zipursky, R.B., Kapur, S., 2000. A voxel-by-voxel analysis of [18F] setoperone PET data shows no substantial serotonin 5-HT(2A) receptor changes in schizophrenia. Psychiatry Res. 99, 123–135.
World Health Organization, 1993. The ICD-10 Classification of Mental and Behavioural Disorders, Diagnostic Criteria for Research.
Yamasue, H., Iwanami, A., Hirayasu, Y., Yamada, H., Abe, O., Kuroki, N., Fukuda, R., Tsujii, K., Aoki, S., Ohtomo, K., Kato, N., Kasai, K., 2004. Localized volume reduction in prefrontal, temporolimbic, and paralimbic regions in schizophrenia: an MRI parcellation study. Psychiatry Res. 131, 195–207.
Yamasue, H., Suga, M., Yahata, N., Inoue, H., Tochigi, M., Abe, O., Liu, X., Kawamura, Y., Rogers, M.A., Takei, K., 2011. Reply to: Neurogenetic effects of OXTR rs2254298 in the extended limbic system of healthy Caucasian adults. Biol. Psychiatry 70, e41–e42.
Yang, Y., Raine, A., Colletti, P., Toga, A.W., Narr, K.L., 2009. Abnormal temporal and prefrontal cortical gray matter thinning in psychopaths. Mol. Psychiatry 14, 561–562 (555).
Yoshida, T., McCarley, R.W., Nakamura, M., Lee, K., Koo, M.-S., Bouix, S., Salisbury, D.F., Morra, L., Shenton, M.E., Niznikiewicz, M.A., 2009. A prospective longitudinal volumetric MRI study of superior temporal gyrus gray matter and amygdala-hippocampal complex in chronic schizophrenia. Schizophr. Res. 113, 84–94.

II. 高齢者によくみられる精神症状の鑑別診断と治療

躁状態

新里和弘　厚東知成

Manic state and manic pseudodementia

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Abstract

The function of each organ including the brain tends to decline with aging. This influences on the appearance of psychotic symptoms in the elderly. A manic state in the elderly is often atypical and different from that in younger and middle aged patients. Manic pseudodementia is an important symptom, which means that elderly manic patients easily show dementia-like behavior because of their Tatendrang (pressured action) or hyperkinesis, so they are misdiagnosed as having dementia. On medication, as the response to drugs is different to that in younger patients, side effects easily appear in the elderly. However, we have very few clinical-pharmacological data on the use of major tranquilizers including anti-manic drugs for elderly patients.

Key words: manic state, elderly, manic pseudodementia, delirium

はじめに

高齢者において，躁状態は決して少なくない病態である．二次性躁病は高齢者に多い[1]ので常に念頭に置くことは当然として，青年・壮年期に躁やうつ病相のエピソードがなく（あるいは病歴を聴取できず），高齢初発の躁病と考えざるをえないケースも存在する．またしばしば高齢者の躁状態は，一見認知症様，あるいはせん妄様にみえる．いわゆる‘躁性仮性認知症（manic pseudodementia）’[2]といわれる状態であり，認知症・せん妄のようにみえてもその背後に躁状態が隠れている場合があることは，留意すべき点でもある．

高齢者躁状態の治療においては，躁状態に対する特異的治療薬である lithium carbonate が使いづらく，他の抗躁剤においても至適用量まで増量させるにあたり時間が必要で，治療が後手に回りやすい面がある．

本稿では，高齢者にみられる躁状態について前半部で鑑別診断を中心にまとめ，後半部で治療を中心に記載を行った．なお高齢者にみられやすい二次性躁病に関しては本稿では触れていないので，他論文[3,4]を参照いただければ幸いである．

1. 高齢者躁状態の鑑別診断

高齢期の躁状態と鑑別診断が重要な疾患として，せん妄と認知症が挙げられる[5]．特に高齢の認知症を伴わないせん妄との鑑別は重要であ

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表1　高齢期の躁病・躁状態の鑑別診断

	高齢の躁病・躁状態 (除；二次性躁病)	高齢者のせん妄 (認知症なし)	認知症[*1]
発病形式	既往の躁(うつ)病の 再燃・悪化[*2]	急性	潜行性
持続・経過	持続性 比較的大きな変動 (寛解もある)	数時間～数週 変動性(夜間の悪化) 無症候期の存在	進行性に悪化
注意・覚醒度	障害されることがある	常に障害される 変動性	通常は保たれる
睡眠	障害される	障害される	通常は保たれる
見当識	しばしば障害される	障害される (変動性)	進行性に低下
記憶	しばしば障害される	障害される (健忘を残す)	進行性に低下
薬物治療	抗躁剤[*3]	抗精神病薬	抗認知症薬

[*1]アルツハイマー型認知症を想定，[*2]高齢初発の躁病・躁状態も当然考慮する必要あり，[*3]副作用の観点から，lithium carbonateより非定型抗精神病薬が第一選択になることも多い．
(文献[5]より改変)

る．せん妄状態は一般的に軽度の意識障害に，幻視や興奮などの異常行動の加わった状態とされるが，特に多動，易怒性，不眠，徘徊などの目立つ'過活動型'と呼ばれるタイプとの鑑別が重要である．

過活動型せん妄状態が躁状態にみえる一方で，高齢者の躁状態も一見するとせん妄類似の病態を呈するので，両者の鑑別が重要となる．十分な観察と周囲の者からの情報聴取が必要である．病歴(以前に躁やうつの波がなかったかどうかなど)，発症の形式(急激な発症はせん妄の可能性が高い)などを参考に鑑別診断を行うが，原因によって使用すべき薬剤が多少異なるため，診断は重要である．認知症も躁状態との鑑別を要するが，後述するようにmanic pseudodementiaと呼ばれる高齢躁状態が認知症様にみえる状態については，特に留意が必要と考える．**表1**に鑑別診断のための表を載せた[5]．

[症例　90歳男性　認知症のないせん妄状態が一見躁状態様にみえた例]

妻は他界しており，長男夫婦，孫との2世帯住居に住む．生来健康で，認知機能の低下も目立たず，句会の役員をしていた．90歳の秋，風邪気味となり，風邪薬を1週間服用したころから精神的な変化が出現した．普段は温和であるのに，夜間ほとんど眠れなくなり，来客があると錯覚し，'来るな来るな'と大騒ぎするようになった．'句会の会長に選ばれた'，'特選を10本とった'など誇大的なことを言い一晩中室内を徘徊．'警察を呼んでくれー'と叫ぶため，救急車と警察が呼ばれた．大騒ぎをした翌朝，家族とともに著者の勤務する病院の外来を初診．ここ数日ほとんど眠れておらず，外来待合室でうつらうつらしている状態であった．記憶は断片的には残存している．風邪薬の中止を指示，ramelteonを処方し，3日間の内服にて症状は完全に消失した．過活動型せん妄による興奮状態と思われた．風邪薬内服による二次性躁病も鑑別としては挙げられるが，軽い意識障害もうかがわれ，風邪薬のせん妄への影響はあったとしても，二次性躁病との関連は薄いと考えられた(二次性躁病は意識障害がないことが原則である)．

2．躁性仮性認知症 (manic pseudodementia)

認知症を伴わないせん妄などが一見すると躁状態類似の症状を呈する場合とは別に，高齢期の躁状態はその症状発現が非典型的となる．一般的に，加齢に伴い疾患の症状の発現パターン

が変わってくることは，我々も日常的に経験することである．精神症状に関していえば，加齢に伴い若年・壮年者でみられやすい幻聴は段々とまれな症状となり，反面，幻視は遭遇しやすい症状となる．また，神経疾患においても典型的神経症候が出にくくなり，精神症状のみが前景に立つ場合がある[6]．

Koenigsbergは，軽い脳疾患を認めていたが最終的には双極性障害(躁状態)と確定診断された症例を報告している．この例は，軽い脳疾患の影響もあり，元来の躁状態が修飾され，あたかも認知症であるかのような様態を呈していた．Koenigsbergは'躁性仮性認知症(manic pseudodementia)'という用語を提唱しているが，この症例の'軽い脳疾患'の部分は，'加齢による脳機能の低下'と置き換えることができると考えられる．つまり，加齢に伴い脳機能の全般的な低下があり，躁状態がまとまりを欠いて，あたかも認知症であるかのような症状を呈するのである．躁病(躁うつ病)をもつ患者が，加齢に伴いその躁症状の出方が変化してくることは当然ありうる．実際の診療においてどのような変化なのかといえば，睡眠障害や行為心拍は壮年期とかわらずにみられる症状ではあるが，健忘が挿入され見当識障害が徐々に目立ってくるため，行動そのものがまとまりを欠くようになる．昔であれば想像もできないようなおかしな行動をする．また身体機能が低下してくるにもかかわらず，行為心拍が持続するため，転倒などの怪我が増え目を離せなくなる．躁状態は様々な精神症状のうちでは，症状発現にエネルギーを要する病態と考えられるが，それゆえ認知面および身体面で予備能力の低下した高齢者では症状発現が非典型的なものとなり，あたかも認知症様となりやすいことは，注意を要する点である．同様のことはせん妄状態にもいえ，'躁性仮性せん妄(manic pseudodelirium)'ともいえる状態がある．ただこの場合には意識障害も混在している状態が一般的と思われる．

[症例　81歳女性　躁性仮性認知症(manic pseudodementia)例]

単身者．もともと精神科に受診歴がある(詳細不明)．子どももいるが独立しており，他県に住む息子が週に1度様子を見にきている．精神科薬の内服は行っていないが，60歳頃から年に数回，非常に活動的で怒りっぽくなる時期があり隣人とのトラブルを繰り返してきた．

80歳を超えたころから，特定の近隣住民の家の前にごみを捨てる，家の前で大きな声を出すことを繰り返し，何度注意されても同じことを繰り返すため，住民から'認知症の人がいて迷惑をかけられている'との連絡が行政にあり，著者の訪問診療に至った．

息子の紹介で室内に招じ入れてくれる．本人は赤のワンピースに真っ赤な口紅で対応は大げさなくらいに親しげである．室内は不潔ではないが物が散乱．落ち着きがなく立ったり座ったりする．話もまとまりに欠けるが，話題は豊富で，昔の自慢話などこちらが制止するまで機嫌よく話し続ける．しかし近隣住民の話については昔のトラブルを引き合いに出し，声が大きくなり興奮する状態であった．同じ話の繰り返しはない．長谷川式で14点，MMSE 11点．集中力の低下に伴い，見当識や短期記憶の点数も低かった．

息子の協力もあり，著者の勤務する病院に通院が開始となった．外来ではolanzapineで薬物調整を行い，眠前の5mgで精神症状は改善．介護保険を受け(要介護1)ヘルパーも導入し，その後の生活は安定した．現在の時点でのHDS-Rは26点，MMSEは28点である．

[症例　81歳男性　せん妄様躁状態を呈した例]

55歳で定年後は，陶芸などの創作活動を行っていた．子ども夫婦は近くに住み，妻との2人暮らしであった．40歳代で抑うつ的となり半年ほど休職したことがあるという．

79歳の秋，昼夜の区別がなくなり，トイレにこもるなどの行動が出現し，家族に伴われてA病院(神経内科)を受診したが，このときは自然と改善したという．80歳の夏，飲酒量が増え昼から飲酒，また20年前の魚釣りの話を，昨日のことのように話し'たくさん釣ってきたから近所に配らないと'といい近隣を訪問したりす

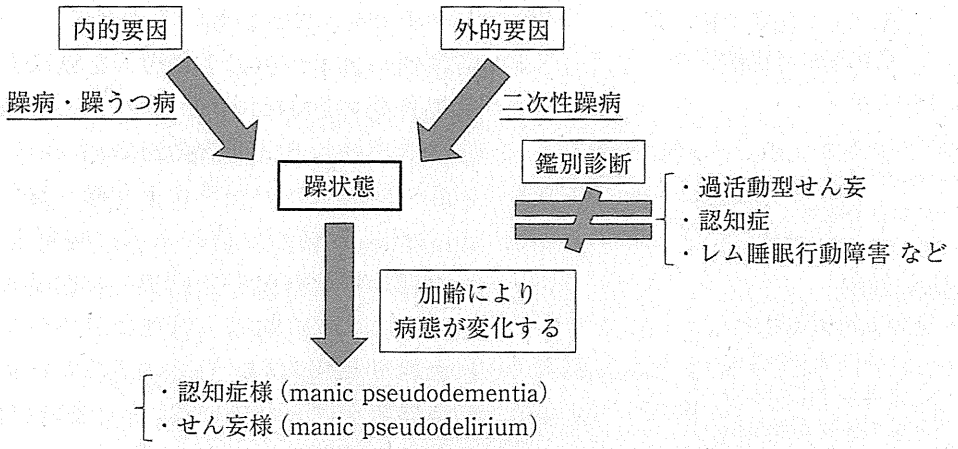

図1　高齢期の躁状態

躁状態という一つの症候群は内的要因(機能性病因)と外的要因の2つの病因をもつ．前者が狭義の躁病・躁うつ病に，後者が二次性躁病に相当する．鑑別診断は本文中に記載のとおりである．また高齢化に伴い躁状態の出方が非典型なものとなる点にも注意が必要である．

る行動がみられるようになった．対応は的外れで，一方的にしゃべり続け，易怒性，強い不眠が認められたという．B精神科病院で入院加療．初診時長谷川式は12点であったが退院時には27点まで改善した．3カ月後，自宅に軽快退院．退院時診断は'せん妄'であった．

退院後B病院に通院していたが，1カ月経った頃から妻への暴言暴力が激しくなった．そのためB病院からの紹介状を持参し，著者の勤務する病院に緊急入院となった．入院時は，とにかく多弁で，妻を'ばあさん'と呼び罵倒する，上機嫌であったかと思うと怒り出す状態であった．入院後は保護室を使用したが，ドア叩きや飛び出し行為が著しく，電動ベッドのコードを首に巻いて'退院させなければ死んでやる'など脅迫したり，'俺は医者だ，この病院を調査するため潜り込んでいるんだ'などの誇大妄想様の言辞が認められた．バルプロ酸の内服に加え，haloperidolの静脈内投与が行われた．徐々に症状は改善し，最終的にはsodium valproate 600 mg，risperidone 2 mg，mianserin 60 mgで安定し，やや抑うつ的に経過した．症状消退後の認知機能は保たれ認知症は否定された．入院時にみられた，睡眠欲求の減少，多弁，自尊心の肥大などから躁うつ病と診断された．40歳代にうつ病相が存在していたことが推測され，その後79歳頃からの一連の変化は，躁うつ病躁状態によるものと思われた．

躁状態ということを中心に再度まとめる．躁状態を引き起こす病因として内的要因(機能的原因)と外的要因とに分けて考えると，前者がいわゆる狭義の躁病(内因性躁病，躁うつ病)であり，後者が二次性躁病である．躁状態と鑑別が重要なものとして，前述したように過活動型のせん妄，認知症，レム睡眠行動障害などが挙げられる．更に高齢者では躁状態が典型的な症状の出方をせず，あたかも認知症様(manic pseudodementia)やせん妄様(manic pseudodelirium)の形で現れやすい．これらの病態を正しく診断することは，その後の治療とも関連することであり重要である．解説図を**図1**に示した．

3. 躁状態の治療

躁病・躁状態の治療について，我が国で使用可能である主な治療薬剤を**表2**に載せた．海外で躁状態の治療に用いられているquetiapineは，2013年3月の時点ではまだ適応となっていない．**表2**にみられるように，多くの躁状態に対する薬剤の発売はあるが，現時点までに高齢者を対象とした検討はほとんどなされていないのが現状である．

lithium carbonateは高齢者には使いにくい．しかし若いときから必須の薬剤として使用して

表2 我が国で使用可能である主な抗躁剤

①炭酸リチウム
②抗てんかん薬
・バルプロ酸ナトリウム
・カルバマゼピン
・ラメルテオン
③抗精神病薬
I. 定型抗精神病薬
・ハロペリドール
・クロルプロマジン
・レボメプロマジン
II. 非定型抗精神病薬
・オランザピン
・アリピプラゾール

いた例，典型的な躁症状を示す例にとっては重要な薬剤である．高齢者では腎機能の潜在的低下があるため，少量でも血中濃度が高値になりやすい．そのため投与量が少量であっても，血中濃度の測定は必須である．甲状腺機能亢進症あるいは電解質異常を伴う患者に対して使用する際には特に注意が必要であり，腎障害を伴うSLEには禁忌とされている．

sodium valproateは高齢者においては，lithium carbonateに比較すると使いやすくしばしば著効する．特に器質性脳病変に基づく躁状態には有効である[7]．注意すべき副作用として肝機能障害があるので，投与前に肝機能のチェックが必要である．acetylsalicylic acid，warfarin，phenytoin，cimetidineなどsodium valproateの血中濃度に影響を与える薬剤の種類が多いので，適宜血中濃度の測定が必要である．

lamotrigineに関しては，2003年に双極性障害の維持療法薬剤としての認可をFDAから受けているが，55歳以上の双極性の患者を対象とした二次解析において，むしろ‘うつ病相’の再発・再燃抑止に有効であった点はlithium carbonateとは対照的である[8]．躁病・躁状態の急性期治療の薬剤としては現時点では適当ではない．

抗精神病薬に関していえば，効果ならびに副作用の観点から従来型(定型)抗精神病薬に比べて非定型抗精神病薬が格段に使いやすい．高齢認知症のBPSDに対する薬物治療では今や非定型抗精神病薬が主流であり，高齢の躁病・躁状態についても同様の治療が望ましいと考える(FDAの勧告[9]は一応頭に置く必要はある)．olanzapineでは50歳以上の双極性障害の患者94例について評価した結果，sodium valproate(divalproex)使用時と比較して効果を認めたとする報告[10]がある．quetiapineは，海外では双極性障害の躁病相(ならびにうつ病相)の適応が承認されているが，前述のように我が国ではまだ未承認である．高齢者を対象にしたquetiapineによる躁病の治験効果の報告[11]があり，これによるとquetiapineは投与4病日目までには有意な改善を認め，高齢の躁病に対する極めて有効な治療薬であることが示されている．高齢者の場合には少量スタートが原則であるが，quetiapineは低力価の薬剤であり，粉薬で処方を行えばごく少量からの処方がしやすい点も利点の一つである．高齢者における非定型抗精神病薬の副作用としては，若年者と変わりはないが，過鎮静や転倒リスクの増大には留意が必要と考えられる．

おわりに

高齢期は臓器の潜在的な機能低下が出現してくる時期であり，それは脳でも例外ではない．精神症状もそれに影響を受ける．高齢者の躁状態が，認知予備能力の低下に伴い典型的ではなくなることは重要な事項である．うつによる仮性認知症だけでなく，‘躁性仮性認知症(manic pseudodementia)’もあることは，頭にとどめておいてよい事態である．また高齢期は，薬剤に対する反応性が成人と異なり副作用が出現しやすい．躁状態を引き起こす原因によって薬物療法は異なり，その反応も成人とは異なってくる．しかし高齢者を対象とした実証的データは明らかに不足している．今後超高齢化社会を迎える我が国において，高齢期の抗躁剤を含む向精神薬の適正使用に関する研究が進展することを期待したい．

文　献

1) Krauthammer C, Klerman GL: Secondary mania: manic syndromes associated with antecedent physical illness or drugs. Arch Gen Psychiatry **35**: 1333-1339, 1978.
2) Koenigsberg HW: Manic pseudodementia: case report. J Clin Psychiatry **45**: 132-134, 1984.
3) 坂元　薫：老年期の躁病・躁状態. 臨床精神医学 **24**: 1287-1292, 1995.
4) 新里和弘：高齢者の躁状態—二次性躁病とその周辺—. 老年精医誌 **22**: 914-919, 2011.
5) 新里和弘：高齢者の躁病・躁状態：鑑別の留意点と2次性躁病. 臨床精神薬理 **15**: 1637-1642, 2012.
6) Daniel SE, et al: The clinical and pathological spectrum of Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy): a reappraisal. Brain **118**: 759-770, 1995.
7) Evans DL, et al: Secondary mania: diagnosis and treatment. J Clin Psychiatry **56**(Suppl): 31-37, 1995.
8) Sajatovic M, et al: Maintenance treatment outcomes in older patients with bipolar I disorder. Am J Geriatr Psychiatry **13**: 305-311, 2005.
9) Center for Drug Evaluation and Research: Deaths with antipsychotics in elderly patients with behavioral distributions[FDA public health advisory]. US Food and Drug Administration, 2005. [http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm053171.htm]
10) Bayer JL, et al: Olanzapine, divalproex and placebo treatment, non-head to head comparisons of older adults acute mania. 10th Congress of the International Psychogeriatric Association. Nice (France), 2001.
11) Sajatovic M, et al: Quetiapine for the treatment of bipolar mania in older adults. Bipolar Disord **10**: 662-671, 2008.

自殺が心配される うつ病が改善！ 100人以上に成果を上げた 食事療法の極意

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血糖コントロールができるとうつ病も改善

糖尿病の患者さんにうつ病が多いことは、以前から知られていました。海外の研究では、糖尿病患者は、そうでない人の2倍以上、うつになりやすいと報告されています。

私が行った調査でも、同じような結果が出ています。糖尿病の治療を受けている患者さんのうち、約37％にうつ症状があり、そのうち8％は治療が必要なうつ病でした。この数は、一般成人の約2倍です。

一方、うつ病患者にも糖尿病が多いことが指摘されています。日本で行われた調査では、成人男性を8年間追跡調査したところ、うつ的な気分の強い人はそうでない人の2・3倍、糖尿病になりやすいという結果が出ています。

このように、うつ病と糖尿病には、相互に深い関係が見られます。

私は以前、内科と連携して、糖尿病患者の不眠症や抑うつ状態といったうつ症状を、治療したことがあります。そのときわかったことは、うつ症状が改善すると、血糖コントロールがよくなり、食事などで血糖コントロールができるようになると、さらにうつ症状も改善することです。

このことから、血糖値の高いうつ病患者に食事指導をすれば、うつ病も改善するのではないかと考えました。そして、うつ病の治療に栄養・食事療法を取り入れるようにしたのです。これまで、100例以上の症例で成果を上げています。その中の、非常に顕著にうつ病が改善した例をご紹介しましょう。

Aさん（60代・女性）は、もともと活発な人で、それまで家事も近所づきあいもうまくこなしていました。ところが、5年前に体調をくずしてから気力がわかなくなり、まともに家事ができなくなったのです。

うつ病と診断され、いろいろな薬を試しましたが、症状は改善せず、半年後に入院。退院後も、はかばかしい改善はありませんでした。一方で、食欲はあり、1日5食も食事をとっていました。

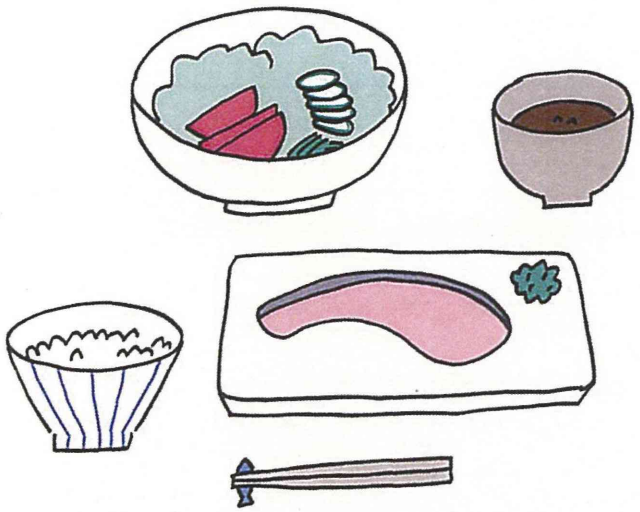
2年前、私が担当医になったときに、Aさんの体重は発病前から20kgもふえていたのです。血糖値、コレステロール値ともに高くて、糖尿病、脂質異常症、肝機能障害が認められました。すぐに、食事療法を開始し、夜の10時には床に就くよう指導しました。

Aさんは食事を3食にへらし、

夜10時に就寝するようになり、薬も規則正しく飲めるようになりました。すると3ヵ月後、洗濯物を自分で干すまでに改善。その後も順調に快方に向かい、半年後には血液データが正常になり、体重もうつ病になる前の値に戻りました。

Aさんのように、ずっと食欲がなかったうつ病患者が食べられるようになると、家族は、「食べられるのはよいこと」と思い、どんどん食べさせます。しかしそれが、逆にうつ病を悪化させてしまうので、注意が必要です。

もう一例は、お酒が好きなBさん（30代・男性）です。もともとやせていましたが、ストレスと飲酒で、3ヵ月で10kg太りました。BMIといって、身長から見た体重の割合を示す指数があります。肥満の手軽な目安で、25以上が肥満になりますが、BさんのBMIは27でした。受診直後はうつ症状が激しく、自殺が心配されるほどだったのです。



カロリー計算はせず、見た目で判断する

Bさんには、まずお酒をやめてもらい、2食だった食事を3食にし、野菜をたくさん食べてもらいました。仕事柄、生活が不規則でしたが、夜10時には寝る生活を指導しました。

その後、数ヵ月で体重が元に戻り、血糖値や中性脂肪値などの血液データが改善して、うつ症状もかなりよくなりました。

こうして、うつ病が改善すると、薬も劇的にへらせます。

ご飯を軽く1膳に主菜と野菜が食事の目安

私たちがうつ病の治療に取り入れている生活指導の目標は、次の3つです。

①3食きちんと食べる
②バランスよく食べる
③夜の10時に就寝する

うつ病患者の多くは、食事のとり方に問題があります。1日2食で済ませたり、一つの食品にこだわって食べたりするのです。結果、食後高血糖になったり、栄養が不足したりします。

ところが、食事療法を取り入れると、生活のリズムが整い、栄養の過不足もなくなってきます。また、服薬もきちんとできるようになるので、相乗的にうつ病が改善していきます。

私といっしょに食事療法に取り組んでいる、管理栄養士の今泉博文（いまいずみひろふみ）さんによると、食事療法のポイントは次の点です。

❶その人に適正な1日の摂取カロリーを、3回に分けてとります。1日2食だった人は、1回の食事量がかなりへります。

❷バランスの目安は、「ご飯を軽く1膳に主菜と野菜」です。カロリー計算はせず、見た目で判断します。スパゲティだけ、ラーメンだけではなく、コンビニの弁当に野菜を一品つける食事のほうが、バランスがとれています。

最初は無理せず、できる範囲でやってもらい、少しずつできることをふやしていくのです。

うつ病には、食事療法を取り入れることが非常に有効です。当センターでは、10日間の「こころと体の健康プログラム」入院を実施しており、心にも体にもよい食事と運動を指導しています。関心のある人は、ぜひご参加ください。

最後に、うつ病治療の基本は薬物療法で、食事、栄養の改善は、あくまで補完的な治療であることを忘れないでください。

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ORIGINAL INVESTIGATION

Impaired cliff avoidance reaction in dopamine transporter knockout mice

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Abstract
Rationale Impulsivity is a key feature of disorders that include attention-deficit/hyperactivity disorder (ADHD). The cliff avoidance reaction (CAR) assesses maladaptive impulsive rodent behavior. Dopamine transporter knockout (DAT-KO) mice display features of ADHD and are candidates in which to test other impulsive phenotypes.
Objectives Impulsivity of DAT-KO mice was assessed in the CAR paradigm. For comparison, attentional deficits were also assessed in prepulse inhibition (PPI) in which DAT-KO mice have been shown to exhibit impaired sensorimotor gating.
Results DAT-KO mice exhibited a profound CAR impairment compared to wild-type (WT) mice. As expected, DAT-KO mice showed PPI deficits compared to WT mice. Furthermore, the DAT-KO mice with the most impaired CAR exhibited the most severe PPI deficits. Treatment with methylphenidate or nisoxetine ameliorated CAR impairments in DAT-KO mice.
Conclusion These results suggest that DAT-KO mice exhibit impulsive CAR behavior that correlates with their PPI deficits. Blockade of monoamine transporters, especially the norepinephrine transporter (NET) in the prefrontal cortex (PFC), may contribute to pharmacological improvement of impulsivity in these mice.

Keywords Dopamine transporter knockout mice · Attention-deficit/hyperactivity disorder · Cliff avoidance reaction · Prepulse inhibition of acoustic startle response · Behavioral inhibition · Impulsivity

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Introduction

Pathological forms of impulsive behavior contribute to the morbidity of numerous psychiatric disorders including attention-deficit/hyperactivity disorder (ADHD) (Barkley 1997; Nigg 2000). Impulsivity has been defined broadly as a lack of behavioral inhibition, including actions that are premature, mistimed and/or, difficult to suppress or control (Dalley et al. 2008; Eagle and Baunez 2010). Impulse control pathologies display actions initiated without due deliberation of other possible options or outcomes. While work in humans has allowed some progress to be made on the pathophysiology of inhibition deficits, mouse models can aid understanding of the brain mechanisms that mediate behavioral inhibition of different sorts. The cliff avoidance reaction (CAR) refers to natural tendency of animals to avoid a potential fall from a height. Under experimental conditions, a test table of a height more than twice the length of the animal is often used. CAR impairment is thought to represent an aspect of maladaptive impulsive behaviors in mature rodents (Matsuoka et al. 2005; Kumakura et al. 2010; Kuroda et al. 2011) that is likely to result from deficient behavioral inhibition. Among the behavioral paradigms used to investigate different forms of impulsive behavior in rodents (Humby and Wilkinson 2011; Winstanley 2011), CAR can provide insights into the underlying neurobiology of behavioral inhibition.

Dopamine transporter knockout (DAT-KO) mice have been suggested to constitute an animal model of ADHD (van der Kooij and Glennon 2007; Arime et al. 2011). Pharmacological and other evidence has long supported roles for alterations in dopaminergic functioning in ADHD (Engert and Pruessner 2008; Tripp and Wickens 2009; Arnsten and Pliszka 2011). DAT-KO mice, produced by transgenic inactivation of the DAT gene, exhibit persistently and profoundly elevated extracellular dopamine (DA) levels in the striatum and nucleus accumbens (Jones et al. 1998; Shen et al. 2004), and somewhat smaller elevations of basal DA levels in the prefrontal cortex (PFC) (Xu et al. 2009). These mice display a variety of behavioral deficits that model aspects of ADHD that include hyperactivity in novel environments and deficits in prepulse inhibition (PPI) (Sora et al. 1998; Gainetdinov et al. 1999; Ralph et al. 2001; Yamashita et al. 2006; Powell et al. 2009; Arime et al. 2012; Uchiumi et al. 2013). Importantly, both hyperactivity and PPI deficits can be ameliorated by treatment with methylphenidate, a first-line psychostimulant medication for ADHD (Gainetdinov et al. 1999; Yamashita et al. 2006). A major relevant action of this drug appears to be its ability to inhibit frontocortical DA uptake via the norepinephrine transporter (NET) (Yamashita et al. 2006; Arime et al. 2012).

We now report use of CAR to assess impulsivity in DAT-KO mice and correlations between individual differences in CAR and PPI. Finally, we evaluate the effects of methylphenidate and the more selective NET blocker nisoxetine on CAR, based on the evidence that each of these drugs can normalize PPI deficits in DAT-KO mice (Yamashita et al. 2006). The novel results of this work indicate that DAT-KO mice manifest a maladaptive impulsive CAR behavior, that individual differences in CAR deficits correlate with those in PPI, and a role for NET blockade in ameliorating these deficits. We discuss the ways in which these data support novel approaches to the treatment of ADHD symptoms.

Materials and methods

Animals

Male wild-type (WT) and homozygous DAT-KO mice (Sora et al. 1998) were bred and maintained on a mixed background combining C57BL/6J and 129Sv/J strains at the Institute for Animal Experimentation in Tohoku University Graduate School of Medicine (Sendai, Japan), which met all Japanese federal government requirements for animal care and use. Offsprings from heterozygote crosses were weaned at 28 days of age and were housed in groups of two to five (segregated by sex) in a temperature and light-controlled colony (lights on at 0800 hours, lights off at 2000 hours), with food and water available ad libitum. Mice from 8–16 weeks of age were used in behavioral tests. All animal experiments were performed in accordance with the guidelines of the Animal Ethics Committee at Tohoku University Graduate School of Medicine (Sendai, Japan).

Drugs

Drugs were dissolved in 0.9 % NaCl solution and were administered intraperitoneally in a volume of 10 ml/kg. Methylphenidate hydrochloride was supplied by Novartis Pharma KK, Japan. Nisoxetine hydrochloride was obtained from Sigma-Aldrich, Japan.

Behavioral tests

Cliff avoidance reaction (CAR)

CAR was assessed using a round wooden platform (diameter, 20 cm; thickness, 2 cm), supported by an iron rod (height, 50 cm) similar to a bar stool (Yoshida et al. 1998). The platform was secured so that the movement of the animal did not affect it. The floor below the platform was carpeted to prevent injury if the animal fell. Ten identical platforms were used for the test.

The test was initiated by gently placing an animal on a platform such that the forelimbs approached its edge. If the animal fell from the platform, it was judged to have impaired CAR. The latency from an initial placement on the platform until falling was recorded. The incidence of impaired CAR was calculated as a percentage index for each group: %(CAR)={the number of intact CAR mice (which did not fall from platforms)/total numbers of tested mice} × 100. Mice which fell from platforms were immediately and gently placed back on the platforms, and the test was continued until 60 min had elapsed. Mice which did not fall from platforms were tested for the same duration of time. Vehicle and drugs were administered prior to CAR test in separate groups of mice. The number of fecal boli was counted at the end of the test. During the testing period, ataxia and stereotypy were also assessed.

Measurement of ataxia and stereotypy

Ataxic behavior was rated at 30 min after the onset of the test session and scored using the numerical rating scale developed by Hiramatsu et al. (1989): (0) inactive or coordinated movements, (1) awkward or jerky movements or loss of balance while rearing, (2) frequent falling or partial impairment of reflexes, (3) inability to move beyond a small area and support of body weight on haunches or abdomen, and (4) inability to move except for twitching movements.

Stereotypy was also rated at 30 min after the onset of the test session according to the numerical rating scale developed by Creese and Iversen (1973): (0) asleep or stationary,

(1) active, (2) predominantly active but with bursts of stereotypic sniffing or rearing, (3) stereotypic activity such as sniffing along a fixed path in the test ground, (4) stereotypic sniffing or rearing maintained in one location, (5) stereotypy in one location with bursts of gnawing or licking, and (6) continual gnawing or licking.

At the end of the session, clasping was also assessed by suspending an animal by its tail (Yamamoto et al. 2000; Cyr et al. 2003). Dyskinetic or dystonic movements of hindlimbs, or a combination of limbs and trunk, were observed every 2 s for 14 s and 1 point was given for each observation of such movements (minimum score 0, maximum score 7).

Prepulse inhibition (PPI)

PPI was tested using four startle chambers (SR-LAB; San Diego Instruments, San Diego, CA) in a sound-attenuated room, as previously described (Yamashita et al. 2006). Each chamber consisted of a nonrestrictive Plexiglas cylinder mounted on a frame inside a ventilated enclosure. Acoustic stimuli and background noise were presented via a high-frequency loudspeaker inside the chamber, mounted above the cylinder. The delivery of acoustic stimuli was controlled by the SR-LAB microcomputer and interface assembly, which also digitized, rectified, and recorded stabilimeter readings, beginning at stimulus onset. Startle magnitude was defined as the average of 65 stabilimeter readings at 1 ms intervals. It was detected and transduced via a piezoelectric device attached to the cylinder's bottom.

PPI test sessions consisted of 64 trials, startle trials (PULSE-ALONE), prepulse trials (PREPULSE+PULSE), and no-stimulus trials (NO-STIM). The PULSE-ALONE trial consisted of a 40-ms 120 dB pulse of broadband noise. The PREPULSE+PULSE trials consisted of a 20-ms noise prepulse, a 100-ms delay, then a 40-ms 120 dB startle pulse (a 120-ms onset-to-onset interval). Prepulse intensities were 3, 6, and 12 dB above the 65 dB continuous background noise. The NO-STIM trials consisted of background noise only. Each test session began and ended with six presentations of the PULSE-ALONE; in between, PREPULSE+PULSE and NO-STIM trials were given 10 times each, and the PULSE-ALONE trials 12 times each in a pseudorandom order. The inter-trial interval was 8–23 s (average 15 s). After the mice were placed in the startle chambers, a 65 dB background noise level was presented for a 5 min prior to initiating the trials, to acclimatize the subjects to the test chamber, and was continued throughout the test session. The initial and final six PULSE-ALONE trials were not included in the analysis in order to ensure the calculation of PPI over a more stable range of startle responses. From these values, two measures were calculated: first, the amount of PPI was calculated as a percentage score for each PREPULSE+PULSE type: %PPI=100−{[(startle response for PREPULSE+PULSE)/(startle response for PULSE-ALONE)]×100}. Second, acoustic startle response (ASR) was calculated as the average response to all of the PULSE-ALONE trials.

Experimental design

Experiment 1 Percent impairment of CAR, stereotypic, ataxic, and clasping behavioral scores were evaluated in drug naïve WT (n=14) and DAT-KO (n=13) mice.

Experiment 2 Percent CAR of drug naïve WT (n=10) and DAT-KO (n=18) mice was assessed along with the latency of the time to the initial fall. On the day following the CAR test, PPI was evaluated.

Experiment 3 Percent impairment of CAR, and stereotypic, ataxic, and clasping behavioral scores, and the number of fecal boli were evaluated in WT (n=20) and DAT-KO (n=20) mice for each drug treatment, with saline, 30 mg/kg methylphenidate, or 30 mg/kg nisoxetine, administered just before start of the testing.

Statistical analysis

The statistical package SPSS for Windows (SPSS Inc., Tokyo, Japan) was used for all data analyses. The effect of genotype on CAR impairment was initially analyzed by the Chi square test. Stereotypic, ataxic, and clasping behavioral scores were analyzed by the Mann–Whitney U test or the Kruskal–Wallis test, followed by the Mann–Whitney U test adjusted by Bonferroni's inequality for multiple comparisons. PPI and ASR data were analyzed by two-way repeated analysis of variance (ANOVA) with GROUP (WT, DAT-KO with intact CAR, and DAT-KO with impaired CAR) as a between-subjects factor, and for PPI prepulse intensity (INTENSITY) as a within-subjects factor. The effects of methylphenidate and nisoxetine on CAR were assessed by Chi Square analysis, while stereotypy scores were subjected to analysis with the Kruskal–Wallis test, and the number of fecal boli to ANOVA with the between subjects factors of GENOTYPE (WT vs. DAT-KO) and DRUG (drug vs. saline). All ANOVAs were followed by the Tukey's HSD test for post hoc comparisons, if applicable. All alpha levels were set at 0.05.

Results

Experiment 1: CAR was impaired in DAT-KO mice

WT mice tended to explore the edge of the platform for several minutes with their snouts, without placing any part

of their bodies substantially over the edge, and then remained still until the end of the test (Fig. 1). By contrast, DAT-KO mice demonstrated marked perseverative hyperlocomotion with bursts of sniffing throughout the test session and repetitive peering-down behavior at the edge of the platform. They often placed substantial portions of their heads and torsos over its edge, even attempting to climb underneath the platform (Fig. 1). This behavior was inherently risky, so that, in the course of this behavior, it was common for these mice to fall from the edge of the platform. About half of the 13 DAT-KO mice tested had impaired CAR during the 60 min observation, while no WT mice displayed impaired CAR (Fig. 2a). This was confirmed by a significant difference between WT and DAT-KO mice (Chi square (1)=8.31; $p<0.01$). In addition, stereotypy scores of DAT-KO mice were significantly higher than those of WT mice ($U=10$; $p<0.001$; Fig. 2b). However, neither WT nor DAT-KO mice showed any significant signs of ataxic or clasping behaviors (ataxia, $U=91$, NS; clasping, U=178, NS; respectively, Table 1).

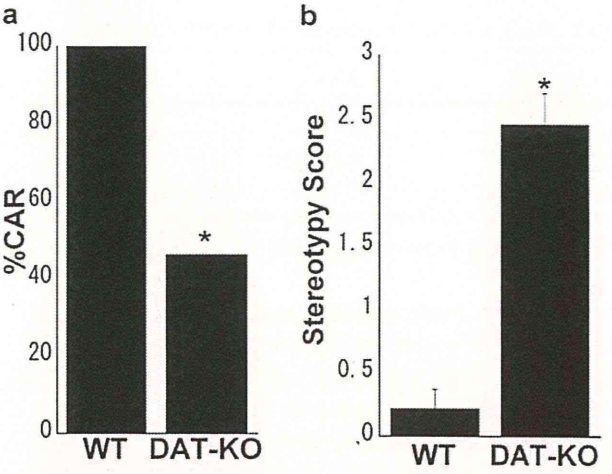

Fig. 2 a) CAR in WT and DAT-KO mice; about half of the DAT-KO mice exhibited impaired CAR during the 60 min observation period. Values represent %CAR. *$p<0.01$, compared with WT mice. b) Stereotypy rating scale in WT and DAT-KO mice; Stereotypy behavioral score in DAT-KO mice was significantly higher than that in WT mice. The stereotypy scores are represented as mean ± SEM. *$p<0.001$, compared with WT mice

Experiment 2: DAT-KO mice with impaired CAR showed severe PPI deficits

In a separate cohort of subjects, the latency to fall in the CAR test was measured in WT and DAT-KO mice. As in the first experiment, about half of the 18 DAT-KO mice fell from the platform within the 60 min test period, but none of the WT mice fell (Fig. 3). The latency to fall in DAT-KO mice was variable, with a normal distribution (mean±SEM=27.1± 12.1; Kormogorov–Smirnov test; $p>0.20$). Subsequently PPI was assessed in all 3 groups, WT mice and the two DAT-KO mice divided into subgroups based on performance in the CAR test: WT mice ($n=10$), all with intact CAR, the DAT-KO mice with intact CAR ($n=9$), and the DAT-KO mice with impaired CAR ($n=9$). ANOVA revealed a significant main effect of INTENSITY on PPI ($F(2, 50)=42.19$; $p< 0.001$), but no interaction of INTENSITY with GROUP ($F(4, 50)=0.18$; NS). The ANOVA did reveal a significant main effect of GROUP ($F(2, 25)=15.52$; $p<0.001$). Post hoc comparisons for each prepulse intensity demonstrated that DAT-KO mice with intact CAR displayed significantly reduced PPI at +12 dB prepulse intensity compared to WT mice, while DAT-KO mice with impaired CAR displayed significantly reduced levels of PPI compared to WT mice across all prepulse intensities (Fig. 4). Furthermore, compared to the DAT-KO mice with intact CAR, the DAT-KO mice with impaired CAR showed significantly impaired PPI at both +6 dB and +12 dB prepulse intensities (Fig. 4). These effects were limited to PPI. For ASR, there was no significant main effect of GROUP ($F(2, 25)=1.46$; NS; Table 2). Thus, all DAT-KO mice had impaired PPI compared to WT mice, but those with impaired CAR had greater disruptions of sensorimotor gating than did the DAT-KO mice with intact CAR.

Experiment 3: methylphenidate and nisoxetine ameliorated CAR impairment in DAT-KO mice

About half of the 20 DAT-KO mice tested with vehicle exhibited impaired CAR during the 60 min observation

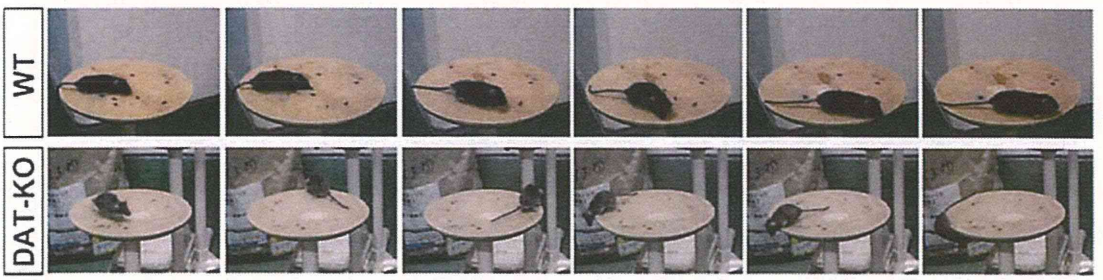

Fig. 1 Sequential photographs of a WT and DAT-KO mouse on the CAR test platforms; Note marked perseverative hyperlocomotion, and bursts of sniffing behavior or repetitive peering-down behavior in the DAT-KO mouse. This DAT-KO mouse finally lost a balance and fell from the platform

Table 1 Ataxia and clasping scores in WT and DAT-KO mice

	WT	DAT-KO
Ataxia Score	0	0
Clasping Score	0.43±0.20	0.38±0.21

Significant ataxic or dyskinetic motor dysfunction was not observed
Ataxia and clasping scores are represented as mean ± SEM

period (Fig. 5a). Methylphenidate reversed the effect of DAT-KO on CAR (Chi square test (3)=8.98; $p<0.05$). The CAR in DAT-KO mice treated with vehicle was significantly impaired in comparison to WT mice treated with vehicle (Chi square (1)= 7.62; $p<0.01$). Methylphenidate treatment did not impair CAR in WT mice (Chi square (1)=1.56; NS), but significantly improved CAR in DAT-KO mice (Chi square (1)=3.96; $p<0.05$). Stereotypy scores also differed significantly between these four groups (H (3)=48.64; $p<0.001$; Fig. 5b). Post hoc analysis of the stereotypy scores found that DAT-KO mice treated with vehicle had significantly higher scores that WT mice treated with vehicle ($U=5.5$; $p<0.01/6$). Methylphenidate significantly increased stereotypy scores in WT mice ($U=3$; $p<0.01/6$), but not in DAT-KO mice ($U=157$; NS). Neither DAT-KO nor WT mice showed any significant signs of ataxic or clasping behaviors after either treatment (ataxia, H (3)=0.00, NS; clasping: H (3)=6.41, NS; respectively, Table 3). The number of fecal boli were decreased after DRUG (methylphenidate) treatment (F(1, 76)=87.96; $p<0.001$). However, there was no significant main effect of GENOTYPE (F(1, 76)=0.11; NS) nor any significant interaction between GENOTYPE and DRUG (F(1, 76)=0.86; NS; Fig. 5c). Methylphenidate significantly reduced the numbers of fecal boli in both WT and DAT-KO mice.

The results of nisoxetine treatment were virtually identical to those of methylphenidate treatment, except on stereotypy.

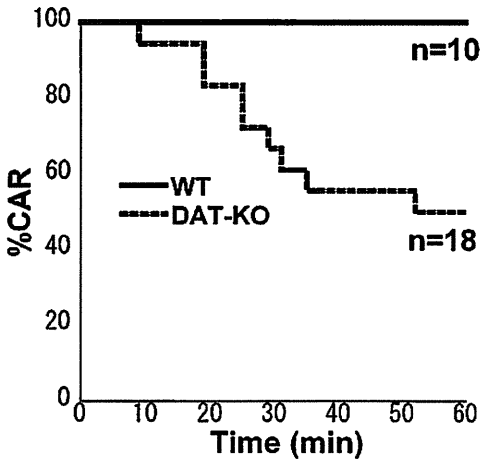

Fig. 3 Time course of CAR impairment in WT and DAT-KO mice; about half of the DAT-KO mice dropped from the platforms within 30 min. Values represent %CAR

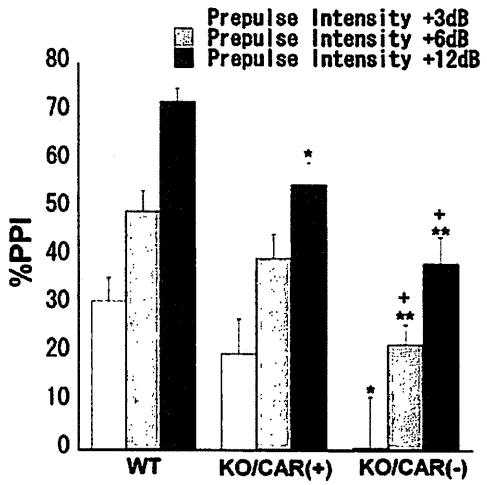

Fig. 4 PPI of WT mice, DAT-KO mice with intact CAR (CAR(+)) and DAT-KO mice with impaired CAR (CAR(−)). PPI of the DAT-KO mice with impaired CAR was significantly decreased compared to that of the DAT-KO mice with intact CAR at the +6 dB and +12 dB prepulse intensities. %PPI Values are represented as mean ± SEM. *$p<0.05$, **$p<0.01$, compared with WT mice; +$p<0.05$, compared with DAT-KO mice with intact CAR

There were thus significant DRUG and GENOTYPE effects on CAR between the four genotype by treatment groups (Chi square test (3)=14.57; $p<0.01$; Fig. 6a). CAR in DAT-KO mice treated with vehicle was significantly impaired compared to WT mice treated with vehicle (Chi square test (1)=7.62; $p<$ 0.01). Nisoxetine had no significant effect on the CAR in WT mice (Chi square test (1)=0.36; NS), but significantly improved the CAR in DAT-KO mice (Chi square test (1)=3.96; $p<0.05$). Stereotypy also differed when all four groups were compared (H (3)=57.46, $p<0.001$; Fig. 6b). Stereotypy was significantly greater in DAT-KO mice treated with vehicle compared to WT mice treated with vehicle ($U=5.5$; $p<0.01/6$), but nisoxetine had no effect on in either group (WT: $U=190$, NS; DAT KO: $U=198$, NS). Neither DAT-KO nor WT mice treated with nisoxetine or vehicle showed any significant signs of ataxic or clasping behaviors (ataxia: H (3)=0, NS; clasping: H (3)=1; Table 4). Nisoxetine reduced the numbers of fecal boli (F(1, 76)=40.82; p<0.001). There was no significant main effect of GENOTYPE (F(1, 76)=0.09; NS) nor a significant DRUG x GENOTYPE interaction (F(1, 76)=0.03, NS; Fig. 6c). Post hoc analyses revealed that nisoxetine

Table 2 Acoustic startle response in WT, DAT-KO mice with intact CAR (CAR(+)) and impaired CAR (CAR(−))

	WT	DAT-KO/ CAR(+)	DAT-KO/ CAR(−)
Acoustic Startle Response	130.8±25.9	73.4±12.9	115.3±30.9

Values (arbitrary unit) are represented as mean startle amplitude±SEM

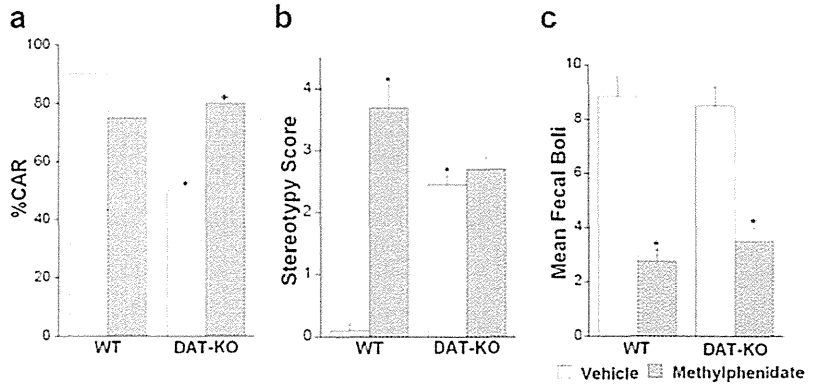

Fig. 5 a) CAR in WT and DAT-KO mice after methylphenidate treatment (30 mg/kg). About half of the DAT-KO mice exhibited impaired CAR during the 60 min observation period in vehicle-treated condition. Treatment of methylphenidate increased CAR in DAT-KO mice. Values represent %CAR. *$p<0.01$, compared with vehicle-treated WT mice; +$p<0.05$, compared with vehicle-treated DAT-KO mice. b) Stereotypy behavior rating scale in WT and DAT-KO mice after methylphenidate treatment (30 mg/kg). Stereotypy scores in DAT-KO mice were significantly higher than those in WT mice in vehicle-treated condition (*$p<0.01$, compared with vehicle-treated WT mice). Stereotypy scores in the drug-treated WT mice were significantly increased (*$p<0.01$, compared with vehicle-treated WT mice). However, stereotypy scores in the drug-treated DAT-KO mice were not significantly altered. Stereotypy scores are represented as mean ± SEM. c) Numbers of fecal boli during the CAR test. The numbers of fecal boli were significantly reduced in the drug-treated WT and DAT-KO mice. The numbers of fecal boli are represented as mean ± SEM. *$p<0.001$, compared with vehicle-treated mice

significantly reduced the numbers of fecal boli in both WT and DAT-KO mice.

Discussion

In this study, we show, for the first time that DAT-KO mice exhibit highly impulsive behavior in CAR testing. DAT-KO mice show perseverative exploration at the edge of the platform with bursts of sniffing. They peer repetitively over the platform edges where they risk falling. The behaviors observed in these mice can be explained by impulsive and stereotypic activation of behavior, consistent with previous studies using other behavioral tests (Spielewoy et al. 2000; Ralph et al. 2001; Pogorelov et al. 2005). Indeed, our results document high levels of stereotypical or repetitive behavior in DAT-KO mice (Fig. 1b) which has been shown to be characteristic of their exploration of novel environments (Ralph et al. 2001). Perseverative patterns of motor behavior in DAT-KO mice that elicit repetitive exploratory behaviors may contribute to the observed CAR impairments. Novelty-seeking may also affect CAR performance since DAT-KO mice are easily aroused by novelty (Spielewoy et al. 2000). Interestingly, DAT knockdown mice have also been shown to exhibit increased exploratory behavior with associated increases in risk-taking behavior (Young et al. 2011). Increased attention to novel stimuli could thus contribute to impulsive behavior in DAT-KO mice as demonstrated in these CAR experiments.

Table 3 Ataxia and clasping scores in WT and DAT-KO mice with methylphenidate (MPD) (30 mg/kg) and vehicle (VEH) treatment

	WT VEH	WT MPD	DAT-KO VEH	DAT-KO MPD
Ataxia Score	0	0	0	0
Clasping Score	0.45±0.20	0	0.30±0.15	0

Significant ataxic or dyskinetic motor dysfunction was not observed. Ataxia and clasping scores are represented as mean±SEM

DAT-KO mice can display anxiety-like responses when first exposed to a novel environment, and perhaps reduced anxiety subsequently (Pogorelov et al. 2005), although his behavior might better be described a perseveration of initial response tendencies, and delayed and prolonged temporal progression of exploratory behavior. However, here we observed differences in the behavioral patterns in the CAR test in DAT-KO mice, characterized by perseverative exploration at the edge to the point of falling, that are not observed in WT mice. Conceivably, such stereotypic exploratory behavior in DAT-KO mice might serve an arousal-reducing effect in these mice, as suggested previously (Pogorelov et al. 2005). It is thus possible that differences in anxiety-like responses could contribute to the CAR impairments observed in DAT-KO mice. It is also conceivable that impaired motor performance observed in DAT-KO mice (Fernagut et al. 2003) could contribute to the observed results. However, there are no obvious motor impairments, ataxic or clasping behaviors in DAT-KO mice (Table 1). It thus seems likely that altered sensorimotor integration, including that assessed by PPI, or impulsivity might underlie at least some of the CAR impairment noted in DAT-KO mice.

DAT-KO mice have long been shown to display impaired sensorimotor gating, as measured by PPI deficits, compared

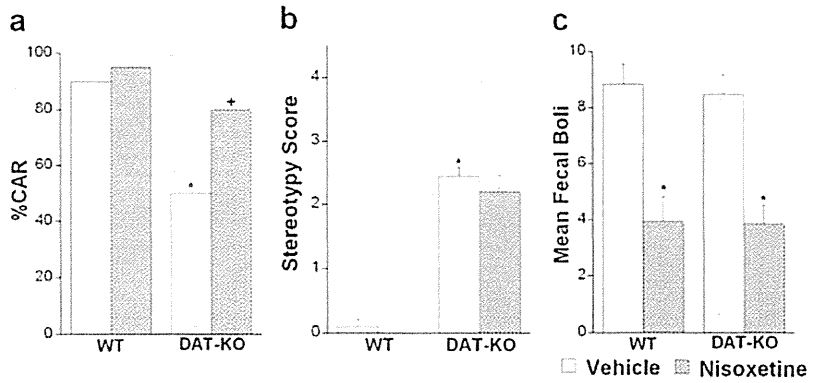

Fig. 6 a) CAR in WT and DAT-KO mice after nisoxetine treatment (30 mg/kg). About half of the DAT-KO mice exhibited impaired CAR during the 60 min observation period in vehicle-treated condition. Treatment of nisoxetine increased CAR in DAT-KO mice. Values represent %CAR. *$p<0.01$, compared with vehicle-treated WT mice; +$p<0.05$, compared with vehicle-treated DAT-KO mice. b) Stereotypy behavior rating scale in WT and DAT-KO mice after nisoxetine treatment (30 mg/kg). Stereotypy scores in DAT-KO mice were significantly higher than those in WT mice in vehicle-treated condition. Drug treatment did not significantly alter stereotypy scores in WT or DAT-KO mice. Stereotypy scores are represented as mean ± SEM. *$p<0.001$, compared with vehicle-treated WT mice. c) Numbers of fecal boli during CAR test. The numbers of fecal boli were significantly reduced in drug-treated WT and DAT-KO mice. The numbers of fecal boli are represented as mean ± SEM. *$p<0.001$, compared with vehicle-treated mice

to WT mice (Yamashita et al. 2006; Arime et al. 2012; Uchiumi et al. 2013). Indeed, the current data demonstrates that DAT-KO mice with impaired CAR exhibit significantly more robust PPI deficits than those with intact CAR (Fig. 4). These results imply that attentional disturbances assessed in the PPI paradigm may contribute, at least in part, to the CAR impairment in DAT-KO mice. It is still possible that DAT-KO mice with intact CAR and more modest PPI deficits may exhibit other forms of impulsivity not detectable in the CAR paradigm. It will be of further interest to determine whether the underlying neural basis for these deficits in DAT-KO mice share some common mechanisms.

Methylphenidate and atomoxetine, a NET blocker used for the treatment with ADHD, are found to be effective in treating impulsivity in the disorder (Aron et al. 2003; DeVito et al. 2009; Wehmeier et al. 2011). Our study demonstrated that impulsivity represented by CAR impairment in DAT-KO mice was ameliorated by treatment with methylphenidate and the selective NET blocker nisoxetine (Figs. 5a and 6a), as was the case for PPI deficits in DAT-KO mice reported previously (Yamashita et al. 2006). With regard to the site of action, the PFC would be a primary candidate, because these drugs increased neuronal activity in this region, but not in the striatum (Bymaster et al. 2002; Koda et al. 2010; Arime et al. 2012). In vivo microdialysis studies have shown that methylphenidate and atomoxetine increase extracellular DA and norepinephrine (NE) levels in the PFC (Bymaster et al. 2002; Berridge et al. 2006; Koda et al. 2010). Uptake via NET is responsible for much of the DA clearance in the PFC (Sesack et al. 1998; Bymaster et al. 2002; Hernandez et al. 2008; Koda et al. 2010); it is thus likely that methylphenidate and nisoxetine activate the prefrontal catecholamine systems by blocking the NET function, thereby helping to improve CAR performance in DAT-KO mice. Methylphenidate can ameliorate some of the hyperactivity in DAT-KO mice as well (Gainetdinov et al. 1999). The locomotor-reducing effects of this drug might also contribute to improvements of CAR impairments. NET blockade has also been shown to modify anxiety-like behavior in mice (e.g. Goddard et al. 2010; Haenisch and Bonisch 2011). Improvement of CAR in DAT-KO mice could also be aided by methylphenidate or nisoxetine effects on emotionality, if this is contributing to CAR impairments.

Table 4 Ataxia and clasping scores in WT and DAT-KO mice with nisoxetine (NSX) (30 mg/kg) and vehicle (VEH) treatment

	WT VEH	WT NSX	DAT-KO VEH	DAT-KO NSX
Ataxia Score	0	0	0	0
Clasping Score	0.45±0.20	0.45±0.17	0.30±0.15	0.20±0.12

Significant ataxic or dyskinetic motor dysfunction was not observed. Ataxia and clasping scores are represented as mean±SEM

Besides the catecholamine systems, altered functioning of the serotonin system has long been implicated in impulsivity (Pattij and Vanderschuren 2008; Eagle and Baunez 2010; Winstanley 2011). In particular, decreased serotonergic neurotransmission has been hypothesized to correlate with diminished inhibitory control and elevated aggression. A previous study has shown that the selective serotonin reuptake inhibitor (SSRI) fluoxetine ameliorated hyperactivity in DAT-KO mice (Gainetdinov et al. 1999). Blockade of the serotonin transporter and activation of serotonergic neurotransmission might also contribute to improvements of deficient behavioral inhibition seen in DAT-KO mice after

some treatments, although they would not apply to the effects of nisoxetine of course.

In conclusion, the present study demonstrated that DAT-KO mice exhibit maladaptive impulsive behavior in the CAR test and that attentional disturbances marked by PPI deficits could in part contribute to this impulsive behavioral deficit. This impulsive phenotype in DAT-KO mice was ameliorated by treatment with methylphenidate and nisoxetine, as has been previously observed for PPI deficits in DAT-KO mice. The effects of these drugs are likely to affect extracellular DA and NE dynamics in the PFC via inhibition of NET, perhaps activating prefrontal catecholaminergic functions. Further analysis of neural circuits involved in CAR will contribute to understanding the pathogenic mechanisms underlying inhibitory control in ADHD, and in helping to identify improved treatments for these symptoms.

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References

Arime Y, Kubo Y, Sora I (2011) Animal models of attention-deficit/hyperactivity disorder. Biol Pharm Bull 34(9):1373–1376. doi:10.1248/bpb.34.1373

Arime Y, Kasahara Y, Hall FS, Uhl GR, Sora I (2012) Cortico-subcortical neuromodulation involved in the amelioration of prepulse inhibition deficits in dopamine transporter knockout mice. Neuropsychopharmacology 37(11):2522–2530. doi:10.1038/npp.2012.114

Arnsten AF, Pliszka SR (2011) Catecholamine influences on prefrontal cortical function: relevance to treatment of attention deficit/hyperactivity disorder and related disorders. Pharmacol Biochem Behav 99(2):211–216. doi:10.1016/j.pbb.2011.01.020

Aron AR, Dowson JH, Sahakian BJ, Robbins TW (2003) Methylphenidate improves response inhibition in adults with attention-deficit/hyperactivity disorder. Biol Psychiatry 54 (12):1465–1468. doi:10.1016/S0006-3223(03)00609-7

Barkley RA (1997) Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychol Bull 121(1):65–94. doi:10.1037/0033-2909.121.1.65

Berridge CW, Devilbiss DM, Andrzejewski ME, Arnsten AF, Kelly AE, Schmeichel B, Hamilton C, Spencer RC (2006) Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. Biol Psychiatry 60(10):1111–1120. doi:10.1016/j.biopsych.2006.04.022

Bymaster FP, Katner JS, Nelson DL, Hemrick-Luecke SK, Threlkeld PG, Heiligenstein JH, Morin SM, Gehlert DR, Perry KW (2002) Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. Neuropsychopharmacology 27(5):699–711. doi:10.1016/S0893-133X(02)00346-9

Creese I, Iversen SD (1973) Blockage of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. Brain Res 55(2):369–382. doi:10.1016/0006-8993(73)90302-8

Cyr M, Beaulieu JM, Laakso A, Sotnikova TD, Bohn LM, Gainetdinov RR, Caron MG (2003) Sustained elevation of extracellular dopamine causes motor dysfunction and selective degeneration of striatal GABAergic neurons. Proc Natl Acad Sci U S A 100(19):11035–11040. doi:10.1073/pnas.1831768100

Dalley JW, Mar AC, Economidou D, Robbins TW (2008) Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. Phamacol Biochem Behav 90(2):250–260. doi:10.1016/j.pbb.2007.12.021

DeVito EE, Balckwell AD, Clark L, Kent L, Dezsery AM, Turner DC, Aitken MR, Sahakian BJ (2009) Methylphenidate improves response inhibition but not reflection-impulsivity in children with attention deficit hyperactivity disorder (ADHD). Psychopharmacol (Berl) 202(1–3):531–539. doi:10.1007/s00213-008-1337-y

Eagle DM, Baunez C (2010) Is there an inhibitory-response-control system in the rat? Evidence from anatomical and pharmacological studies of behavioral inhibition. Neurosci Biobehav Rev 34 (1):50–72. doi:10.1016/j.neubiorev.2009.07.003

Engert V, Pruessner JC (2008) Dopaminergic and noradrenergic contributions to functionality in ADHD: the role of methylphenidate. Curr Neuropharmacol 6(4):322–328. doi:10.2174/157015908787386069

Fernagut PO, Chalon S, Diguet E, Guilloteau D, Tison F, Jaber M (2003) Motor behaviour deficits and their histopathological and functional correlates in the nigrostriatal system of dopamine transporter knockout mice. Neuroscience 116(4):1123–1130. doi:10.1016/S0306-4522(02)00778-9

Gainetdinov RR, Westel WC, Jones SR, Levin ED, Jaber M, Caron MG (1999) Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science 285(5400):397–401. doi:10.1126/science.283.5400.397

Goddard AW, Ball SG, Martinez J, Robinson MJ, Yang CR, Russell JM, Shekhar A (2010) Current perspectives of the roles of the central norepinephrine system in anxiety and depression. Depress Anxiety 27(4):339–350. doi:10.1002/da.20642

Haenisch B, Bonisch H (2011) Depression and antidepressants: insights from knockout of dopamine, serotonin or noradrenaline re-uptake transporters. Pharmacol Ther 129(3):352–368. doi:10.1016/j.pharmthera.2010.12.002

Hernandez LF, Segovia G, Mora F (2008) Chronic treatment with a dopamine uptake blocker changes dopamine and acetylcholine but not glutamate and GABA concentrations in prefrontal cortex, striatum and nucleus accumbens of the awake rat. Neurochem Int 52(3):457–469. doi:10.1016/j.neuint.2007.08.005

Hiramatsu M, Cho AK, Nabeshima T (1989) Comparison of the behavioral and biochemical effects of the NMDA receptor antagonists, MK-801 and phencyclidine. Eur J Pharmacol 166(3):359–366. doi:10.1016/0014-2999(89)90346-4

Humby T, Wilkinson LS (2011) Assaying dissociable elements of behavioural inhibition and impulsivity: translational utility of animal models. Curr Opin Pharmacol 11(5):534–539. doi:10.1016/j.coph.2011.06.006

Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, Caron MG (1998) Profound neuronal plasticity in response to