Table 4
Studies included in meta-analysis for rs6295.

Author	Yesr	Ethnic	Diagnostic system	N[a]			C allele[a]			G allele[a]		
				SCZ	BP	CON	SCZ	BP	CON	SCZ	BP	CON
Kishi		Asian	DSM-IV	857	1028	1810	1036	1475	2726	392	581	894
Huang	2004	Caucasian	DSM-IV	108	88	107	86	81	118	130	95	96
Sullivan	2009	Caucasian	DSM-IV	-	32	47	-	31	57	-	31	35

a BP: bipolar disorder SCZ: schizophrenia CON: controls.

panic disorder (Strobel et al., 2003) and antidepressant response in major depressive disorder (MDD) (Lemonde et al., 2004; Serretti et al., 2004; Arias et al., 2005; Hong et al., 2006; Parsey et al., 2006; Yu et al., 2006). Recently, we reported that *HTR1A* was associated with MDD in a meta-analysis (Kishi et al., 2009b). In addition, we reported that *HTR1A* was associated with Japanese methamphetamine-induced psychosis patients (Kishi et al., 2009c). Our previous studies selected the same variant as this study. Huang and colleagues reported that rs6295 was associated with schizophrenia (Huang et al., 2004). However, we could not replicate the association between rs6295 and schizophrenia found in their study (Huang et al., 2004). We also found no association between rs6295 and schizophrenia in the meta-analysis. Recent studies reported that rs6295 was associated with improvement in negative symptoms from anti-psychotics such as risperidone (Reynolds et al., 2006; Wang et al., 2008; Mossner et al., 2009) and that 5-HT1A receptor agonists such as tandospirone produced improvements in cognitive impairment in schizophrenia (Sumiyoshi et al., 2001a, 2007; Meltzer and Sumiyoshi, 2008). We assume that quantitative traits, including negative symptoms and cognitive symptoms for schizophrenic patients, will be key features in assessing the genetic contribution of *HTR1A* to schizophrenia.

The heterogeneity in this meta-analysis for schizophrenia may have resulted from: (1) different ancestries (Asian population vs. Caucasian population), (2) the small size of the overall sample included in the meta-analysis (851 schizophrenic patients and 911 control subjects) and (3) the inclusion of different samples in the screening method in the meta-analysis (Shi et al., 2008).

A few points of caution should be mentioned with respect to our results. First, our results in the case-control study and meta-analysis may be due to biased samples, such as unmatched age and gender samples, or to small sample sizes such as case-control genetic association studies (Shi et al., 2009). Therefore, the significant associations between *HTR1A* and BP in the case-control study and meta-analysis may be due to type I errors. However, we performed a logistic regression analysis to compare the phenotypes of each of the examined SNP genotypes, using several clinical factors as other independent variables to adjust for possible confounding. Second, we did not perform a mutation scan of *HTR1A*. Because we consider it to be difficult to evaluate the association of such extremely rare variants from the viewpoint of statistical power, a replication study using a larger sample is required for conclusive results. Third, we did not include GWAS data regarding rs6295 in this meta-analysis. Fourth, we combined Asian and Caucasian populations in this meta-analysis. However, we did not detect significant heterogeneity for BP samples. In addition, we included the different samples of the screening method in the meta-analysis.

5. Conclusion

In conclusion, our results suggest that *HTR1A* may play a role in the pathophysiology of Japanese BP, but not Japanese schizophrenia. Further, in the meta-analysis, *HTR1A* was associated with BP patients.

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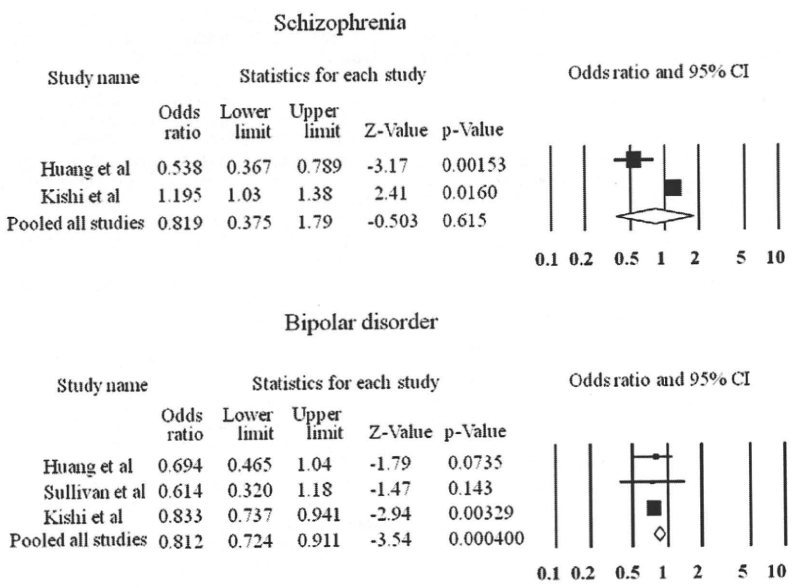

Fig. 1. Forest plots of OR with 95% CI for rs6295.

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ORIGINAL INVESTIGATION

Cabergoline, a dopamine receptor agonist, has an antidepressant-like property and enhances brain-derived neurotrophic factor signaling

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Abstract
Rationale Dopamine agonists have been implicated in the treatment of depression. Cabergoline is an ergot derivative with a high affinity to dopamine D_2-like receptors; however, there have been few preclinical studies on its antidepressant-like effects.
Materials and methods Behavioral effects of cabergoline were examined in rats using forced swimming (FST), novelty-suppressed feeding (NST), open field (OFT), and elevated-plus maze (EPT) tests. In a single treatment paradigm, behaviors of rats were analyzed 4 h after single injection of cabergoline (s.c., 0–4 μmol/kg). In a repeated-treatment paradigm, OFT, EPT, and FST were conducted on days 11, 12, and13–14, respectively, during daily cabergoline injections (s.c., 0.5 μmol/kg), and then hippocampus was removed 24 h after the last injection. NST was conducted in a separate experiment at day 14. Western blotting was used for the analysis of the protein levels of brain-derived neurotrophic factor (BDNF) and the activation of intracellular signaling molecules.
Results Single injection of cabergoline demonstrated decreased immobility in FST and distance traveled during 0–10 min in OFT, while time spent and entry into open arms were increased at 4 μmol/kg. When cabergoline was repeatedly administered, immobility in FST and the latency of feeding in NSF were significantly reduced, while vertical movement was increased in OFT. The time in closed arms was tended to be decreased in EPT. Expression of BDNF and activation of extracellular signal-regulated kinase 1 were up-regulated after the chronic administration of cabergoline.
Conclusions Cabergoline exerts antidepressant- and anxiolytic-like effects, which may be mediated by potentiation of intracellular signaling of BDNF.

Keywords Antidepressant · Anxiety · Brain-derived neurotrophic factor (BDNF) · Cabergoline · Depression · Dopamine receptor agonist · Extracellular signal-regulated kinase (ERK) · Locomotor activity

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Introduction

The current first-line treatments of depressive disorder are serotonin-selective reuptake inhibitors (SSRI), serotonin–noradrenalin reuptake inhibitors (SNRI), and tricyclic antidepressants; however, a substantial proportion of depressed patients are refractory to such treatments (Keller 2005). Dopamine receptor agonists have been thought as one of the promising candidates to improve outcomes of patients with treatment-resistant and nonremitting depression (Dunlop and Nemeroff 2007). Cabergoline is an ergot

derivative and dopamine D_2 receptor-like agonist with a lower affinity to D_1-like, adrenergic, and serotonergic receptors (Millan et al. 2002). Its agonistic effect on dopamine receptors has been utilized in therapies for Parkinson's disease and hyperprolactinaemia. Depression is one of the common complications in Parkinson's disease (Yamamoto 2001), and decreased dopamine transmission has been suggested as one of the causes of this phenomenon (Lemke 2008). Indeed, dopamine receptor agonists such as pramipexole and pergolide demonstrated their effectiveness both on depression and motor functioning in patients with Parkinson's disease (Rektorová et al. 2003; Lemke et al. 2005, 2006; Leentjens et al. 2009). Patients with treatment-resistant depression were also subjected to administration of dopamine receptor agonist in addition to contemporary antidepressants, and favorable results were reported with regard to pramipexole (Lattanzi et al. 2002), bromocriptine (Inoue et al. 1996), and pergolide (Izumi et al. 2000). Takahashi et al. (2003) reported that cabergoline was effective in two cases of refractory depression as a supplementation therapy to an SNRI milnacipran. However, detailed mechanism underlying antidepressant-like effects of dopamine receptor agonists including cabergoline is still unclear.

Brain-derived neurotrophic factor (BDNF) is one of the neurotrophins and thought to play an important role both in the etiopathology of depression and the action of antidepressants (Castrén et al. 2007; Duman and Monteggia 2006; Adachi and Kunugi 2008). BDNF binds to its high-affinity receptor TrkB and low-affinity receptor p75 (Huang and Reichardt 2001) and exerts its biological effects through various intracellular pathways including ERK and inositol trisphosphate kinase (PI3K)-Akt signalings (Huang and Reichardt 2001; Kaplan and Miller 1997). BDNF is suggested to be involved in neuronal differentiation, survival, and synaptic plasticity (Poo 2001). Recently, we reported that its important roles in the regulation of synaptic transmission in cultured hippocampal and cortical neurons (Kumamaru et al. 2008; Numakawa et al. 2009). Changes in the expression of BDNF in patients with depression have been reported in the hippocampus, which is one of the important regions in mood regulation. For example, mRNA and protein levels of BDNF were decreased in the postmortem hippocampus of depressive patients (Dunham et al. 2009) and animal models of depression (Grønli et al. 2006). Treatment with antidepressants up-regulates the expression of BDNF in the hippocampus of patients with depression (Chen et al. 2001) and animals (Nibuya et al. 1995). The BDNF up-regulation was also observed after other antidepressive treatments including chronic electroconvulsive therapy (Nibuya et al. 1995). These studies suggest that BDNF may be involved in the final common pathway in the action of the antidepressive therapies.

Dopamine signaling may play a significant role in the regulation of BDNF expression. Dopamine receptor agonists are shown to increase the BDNF levels in cultured astroglial (Ohta et al. 2003) and neuronal cells (Küppers and Beyer 2001; Du et al. 2005). In contrast, haloperidol, an antipsychotic drug which antagonizes D_2 receptor, decreased the expression of BDNF in the brain (Angelucci et al. 2000; Dawson et al. 2001), although some atypical antipsychotics were reported to increase BDNF levels (Fumagalli et al. 2004; Parikh et al. 2004). Ohta et al. (2004) found that cabergoline induced BDNF up-regulation in cultured astrocytes. However, it is unclear whether administration of cabergoline induces the expression of BDNF in vivo.

These previous studies prompted us to test the hypothesis that cabergoline has an antidepressant-like property that is mediated through enhanced BDNF expression in the hippocampus. Here, we investigated the effects of acute/chronic systemic cabergoline administration in the behavioral tests, which are useful for screening of antidepressants and anxiolytics [i.e. forced swim test (FST), novelty-suppressed feeding test (NST), open field test (OFT), and elevated-plus maze test (EPT)]. Antidepressant-like effect was also analyzed using Wistar-Kyoto rats, an innate animal model of depression (Paré 1989). Furthermore, we tried to elucidate the possible molecular mechanism underlying the action of cabergoline by examining changes in the expression of BDNF, TrkB, and p75, as well as activation of ERK and Akt, downstream signalings of TrkB.

Materials and methods

Animals and experimental design

All the experimental procedures were approved by the ethics review committee for animal experimentation at the National Institute of Neuroscience, Japan and done with every effort to minimize the number of animals used and their sufferings. Male Wistar and Wistar-Kyoto rats were purchased from Charles River Japan (Yokohama, Japan) at 6 weeks old and housed in standard laboratory condition (22–24°C, 40–60% humidity, 3:00 PM light on, 3:00 AM light off). Rats were kept in polycarbonate cages in groups, and laboratory chaws and water were available ad libitum. All rats were handled daily for a few minutes from 7 weeks of age. Cabergoline (Mylan Pharmaceutical, Tokyo, Japan) and fluvoxamine (Meiji-seika, Tokyo, Japan) were dissolved in 0.5% carboxymethyl cellulose (Sigma, St. Louis, MO, USA) in sterilized water. In the acute treatment paradigm, behaviors of rats were analyzed 4 h after single injection (from 8:00 to 10:00 AM) of cabergoline (0, 0.25, 0.5, 1, 2, and 4 μmol/kg BW, s.c.) or 1 h after fluvoxamine treatment (138 μmol/kg BW, p.o.). In the chronic treatment

paradigm, OFT, EPT, and FST were conducted at days 11, 12, and 13–14, respectively, during repeated injections of cabergoline (s.c. s.i.d. at 3:00PM, 0.5 μmol/kg BW). The hippocampus was removed from the rat 24 h after the last injection of cabergoline in the chronic treatment regimen. The sample was quickly frozen on dry ice after the removal and stored at −80°C until used. NST was conducted at day 14 of the chronic treatment regimen in a separate experiment. The doses of cabergoline were according to a previous study on an animal model of Parkinson's disease (Miyagi et al. 1996). The schedule of repeated administration was chosen because 2 weeks are needed for antidepressants to change behaviors in several animal models of the depression (Gambarana et al. 2001).

Behavioral tests

FST A modified version of FST (Detke et al. 1995), which consisted of two swim sessions, was carried out. In the first swim session, the rat was introduced into a plastic cylinder (40 cm depth, 20 cm diameter) filled with 25 cm deep water of 23–25°C and forced to swim for 15 min. Twenty-four hours after the first session, the rat was reintroduced into the same cylinder, and their 5-min swimming was observed. The behavior of the rat was recorded on videorecorder (SONY, Tokyo, Japan). After each swim session, the rat was removed from the cylinder, dried with paper towels, placed in the resting cages for 20 min, and then returned to its home cage. Water in the cylinder was renewed between sessions.

Analysis was done on data from the second session of the FST in the acute, and day 14 (FST) in the chronic treatment regimen. Behavioral measures of the rat were defined as follows: (1) immobility—floating in the water without active moving of its limbs and making only slight movements necessary to keep its head above water; (2) swimming—moving more than necessary to keep its head above water and its forelimbs being in the water; (3) climbing—actively trying to climb the wall of the cylinder with its forelimbs above the water; (4) diving—diving into water and its entire body being submerged. The time was manually recorded when each one of the behaviors had started. The time a rat displayed one of the behaviors was calculated by subtraction of the start time from the end time of the behavior.

NST Rats were deprived from food for at least 16 h before the experiment and introduced into an open-field apparatus (100 cm×100 cm×40 cm) with the rat chow on the filter paper placed in the center of the field. Behavior was recorded from the charged-coupled device (CCD) camera above the field. Latency of feeding was measured manually. If the feeding did not happen within 10 min, the latency was recorded as 10 min.

OFT Voluntary movements during 30-min test were monitored by introducing the rat into the open-field apparatus using a CCD camera, and images were captured on Macintosh computer by the Image OF software (modified software based on the NIH image program developed at the U.S. National Institute of Mental Health; modified by O'Hara & Co., Tokyo, Japan). Distance traveled and time spent in the central square that was enclosed by the peripheral zone 20 cm from the wall were automatically calculated by the Image OF software. Rearing and grooming behaviors were also recorded.

EPT Elevated-plus maze has two closed arms that have 50-cm high walls around the arm (10 cm width, 50 cm length; east and west) and two open arms that have 0.5-cm ridges around the arms (10 cm width, 50 cm length; north and south). The maze was elevated 40 cm from the floor. The rat was introduced into the eastern arm of the closed arms and allowed to move freely for 15 min. Behaviors were monitored with a CCD camera and recorded on the Macintosh computer with the Image-EP software (modified software based on the NIH image program developed at the U.S. National Institute of Mental Health; modified by O'Hara & Co., Tokyo, Japan). Total duration of the time spent and entries into each arms were obtained with the Image-EP software. Rearing and grooming behaviors were also recorded.

Behaviors were assessed by a rater who was blind to the cabergoline- or vehicle-treatment status of each rat.

Western blotting

The separated hippocampus was homogenized in lysis buffer, and the protein concentration in the sample was determined before the western blotting assay as previously reported (Numakawa et al. 2003, 2004, 2009). The equivalent amounts of total protein were assayed for each immunoblotting. Primary antibodies were used at the following dilutions: anti-Akt (1:1,000, Cell Signaling, Danvers, MA, USA), anti-pAkt (1:1,000, Cell Signaling), anti-ERK (1:1,000, Cell Signaling), anti-pERK (1:1,000, Cell Signaling), anti-p75 (1:1,000, Promega, Madison, WI, USA), anti-TrkB (1:1,000, BD Biosciences, San Jose, CA, USA), and anti-BDNF (1:200, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) antibodies.

Data analysis

All data were expressed as mean ± standard error (SEM). Behavioral data were analyzed with repeated analysis of variance (ANOVA) or one-way ANOVA, followed by post hoc Student's *t* test or Dunnett method if appropriate. Data obtained from western blotting were analyzed by Student's

t test. Outliers were removed if the Smirnov–Grubbs test was significant. The R software (version 2.7.2, R Foundation for Statistical Computing, Vienna, Austria) was used for the statistical analysis. The statistical significance was considered when p value was less than 0.05.

Results

Behavioral effects of single cabergoline administration

In FST, Wistar rats showed more active climbing as the dose of the drug increased [$F(1,38)=6.9$, $p=0.013$; Fig. 1a] and exhibited significantly longer duration of climbing at the doses of 1.0 (167±23 s; $t=3.5$, $p=0.005$) and 2.0 μmol/kg BW (154±16 s; $t=2.9$, $p=0.022$) in comparison with the vehicle-treated group (87±13 s). The total duration of swimming was not significantly different after treatment with any dose of cabergoline [$F(1,38)=1.0$, $p=0.32$]. The cabergoline-treated rats showed significantly less immobility compared with that of the vehicle-treated group in a dose-dependent manner [$F(1,38)=15.3$, $p<0.001$; 75±14 s, $t=2.6$, $p=0.045$ at 0.5 μmol/kg; 42±12 s, $t=4.2$, $p<0.001$ at 1.0 μmol/kg; 39±13 s, $t=4.3$, $p<0.001$ at 2.0 μmol/kg]. In addition, the latency of immobile posture was increased in a dose-dependent fashion [$F(1,38)=4.9$, $p=0.032$] and significantly longer at 1.0 μmol/kg (160±23 s, $t=3.0$, $p=0.017$; Fig. 1b) than vehicle treatment (59±12 s). A few rats (3/40) displayed diving, and no dose-dependent response was observed.

As expected, Wistar-Kyoto rats showed significantly shorter climbing [$F(2,1,46)=75$, $p<0.001$; Fig. 1c] and swimming [$F(2,1,46)=35$, $p<0.001$], and longer immobility [$F(2,1,46)=174$, $p<0.001$] in comparison with Wistar rats. Cabergoline treatment in Wistar-Kyoto rats demonstrated significantly increased climbing (28±8 s; $t=2.4$, $p=0.047$) and a trend for reduced immobility (217±10 s; $t=2.0$, $p=0.097$), although no significant changes in immobility ($p=0.49$) were observed in fluvoxamine-treated group. In Wistar rats, reduction in the immobility by cabergoline (61±16 s, $t=3.1$, $p=0.01$) and a trend of the same direction by fluvoxamine (78±26 s, $t=2.1$, $p=0.08$) were observed.

In NST, non-significant effect was shown in the Wistar rats after single cabergoline administration on the latency of feeding in the novel environment (597±3 s in vehicle, 526±52 s in cabergoline group; $t=1.4$, $p=0.22$; Fig. 1d).

In OFT, Wistar rats were treated with cabergoline at three doses (0, 0.25, and 1.0 μmol/kg BW), and its behavior was monitored for 30 min. Distance traveled (horizontal movement; Fig. 2a) and the number of rearing (vertical movement; Fig. 2b) decreased as the time passed [$F(5,10,75)=27.3$; $p<0.001$ for distance; $F(5,10,75)=31.4$, $p<0.001$ for rearing]. There was an interactive effect between dose and time [$F(5,10,75)=2.66$, $p=0.008$], and distance was decreased in the 1.0 μmol/kg BW dose group during 0–5 (9.1±2.1 m, $p=0.037$) and 5–10 min (7.1±1.2 m, $p=0.023$) periods compared with the vehicle-treatment group (16.5±2.1 m, 13.1±1.9 m during 0–5 and 5–10 min periods, respectively). However, no significant effect of cabergoline was observed

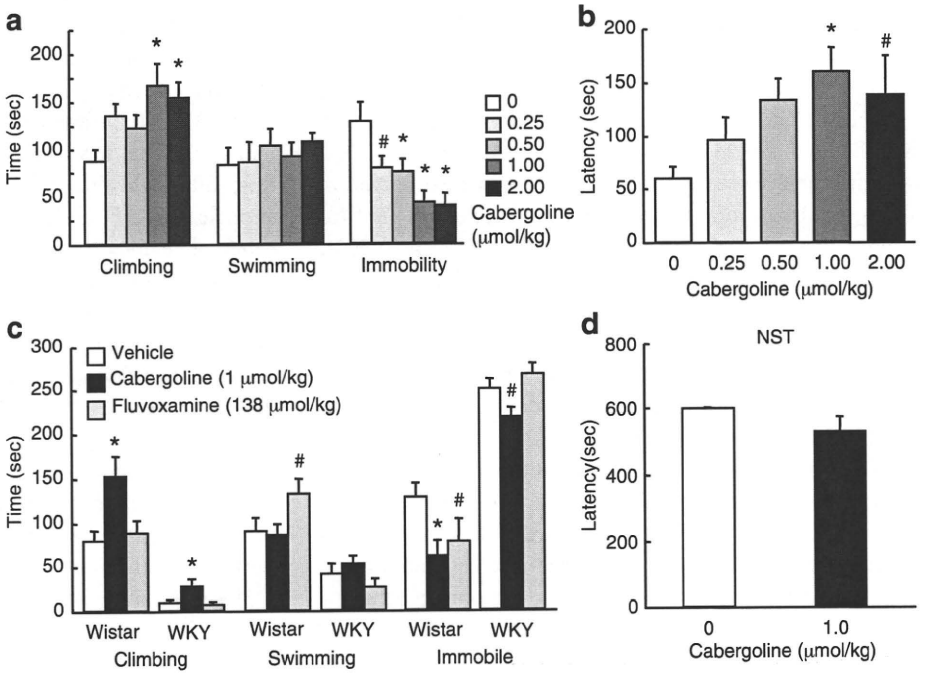

Fig. 1 Effects of single administration of cabergoline observed in FST and NST **a** duration of climbing, swimming, and immobility in Wistar rats in FST ($n=8$); **b** latency of immobile posture in Wistar rats in FST; **c** duration of climbing, swimming, and immobility in Wistar and Wistar-Kyoto (WKY) rats after cabergoline or fluvoxamine treatment in FST ($n=7$–10); **d** latency of feeding of Wistar rats in NST ($n=8$). *Columns* and *bars* represent mean±SEM. *$p<0.05$, #$p<0.1$ vs. vehicle

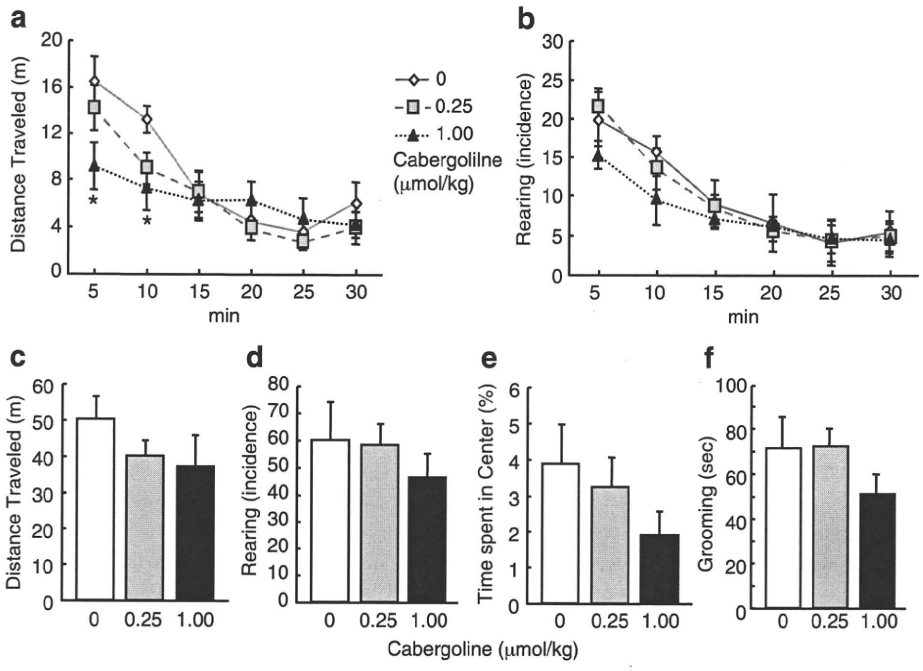

Fig. 2 Effects of single administration of cabergoline of different doses observed in OFT **a** time course curves of distance traveled for rats receiving different doses of cabergoline, **b** the number of rearing, **c** total distance traveled, **d** total number of rearing, **e** time spent in center, and **f** grooming. *Symbols/columns* and *bars* represent mean±SEM. $n=6$ for each group. *$p<0.05$ vs. vehicle

in the total distance traveled during the 30-min test [$F(1,16)=1.5$, $p=0.24$; Fig. 2c]. Cabergoline had no significant effect on total number of rearing [$F(1,16)=1.0$, $p=0.32$; Fig. 2d], time spent in center [$F(1,15)=0.13$, $p=0.72$; Fig. 2e], or grooming behaviors [$F(1,16)=0.51$, $p=0.54$; Fig. 2f].

In EPT, the cabergoline-treated animals (4 μmol/kg BW) spent more time in open arms (163±45 s for cabergoline vs. 61±13 s for vehicle, $t=3.2$, $p=0.008$; Fig. 3a) and less time in closed arms (515±82 s for cabergoline vs. 744±30 s for vehicle, $t=3.6$, $p=0.003$) compared with the vehicle control. The cabergoline treatment slightly, but not significantly, reduced the number of entries into open arms at two doses (1.4±0.4 and 2.2±0.5 at 0.25 and 1 μmol/kg, respectively; Fig. 3b). A remarkable increase in open arm-entry was observed in the 4 μmol/kg group compared with vehicle (9.4±3.0 for cabergoline vs. 3.2±0.5 for vehicle, $t=3.2$, $p=0.007$). There was a significant interaction between time and doses on distance traveled [$F(1,1,76)=5.1$, $p=0.027$; Fig. 3c]. Clear reductions in the distance traveled were observed in cabergoline-treated group from 0 to 5 ($t=2.5$ $p=0.047$ in 0.25 and $t=3.1$ $p=0.011$ in 1 μmol/kg) and from 5 to 10 min ($t=2.6$, $p=0.035$ in 1 μmol/kg). Total distance [$F(1,36)=0.81$, $p=0.37$; Fig. 3f] and the number of rearing were not different between groups [$F(1,36)=0.28$, $p=0.60$; Fig. 3d, g]. There was a trend toward reduced percentage of entries into open arms at 0.25 μmol/kg ($t=2.1$, $p=0.09$; Fig. 3e). A decrease in grooming behavior was also observed at 4 μmol/kg group ($t=4.3$, $p<0.001$; Fig. 3h).

Behavioral effects of chronic cabergoline administration

In FST, there were significant reduction in immobility (99±14 for cabergoline vs. 143±15 s for vehicle; $t=-2.3$, $p=0.035$; Fig. 4a) and significant increase in swimming (116±14 for cabergoline vs. 71±9 s for vehicle; $t=3.0$, $p=0.01$). Latency of the immobile behavior was not significantly different between the two groups ($t=-0.15$, $p=0.88$; Fig. 4b).

In NST, Wistar rats demonstrated a significant reduction in the latency of feeding in the novel environment after the chronic administration of cabergoline (562±38 s in vehicle, 395±63 s in cabergoline group; $t=2.3$, $p=0.04$; Fig. 4c).

In OFT, the cabergoline-treated rats showed slightly longer distance traveled compared with the vehicle-treated rats, although the difference did not reach statistical significance [$F(1,14)=1.0$, $p=0.33$; Fig. 5a, c]. The cabergoline treatment also induced significant increase in the number of vertical movements [$F(1,14)=8.9$, $p=0.01$; Fig. 5b]. Total number of rearing was increased after the treatment (94±10 vs. 55±9; $t=3.0$, $p=0.01$; Fig. 5d). No significant differences were observed in the time spent in the center area ($t=1.2$, $p=0.29$; Fig. 5e) or grooming ($t=-0.85$, $p=0.41$; Fig. 5f).

In EPT, cabergoline-treated rats demonstrated trends toward a decrease in the time in closed arms ($t=-2.0$, $p=0.071$; Fig. 6a), as well as an increase in the time in center ($t=1.7$, $p=0.096$) and total frequency of entry ($t=2.1$, $p=0.051$; Fig. 6b). There was a tendency of more active locomotion during the 15-min test [$F(1,14)=3.3$, $p=0.09$; Fig. 6c, f]. No significant difference was found in the

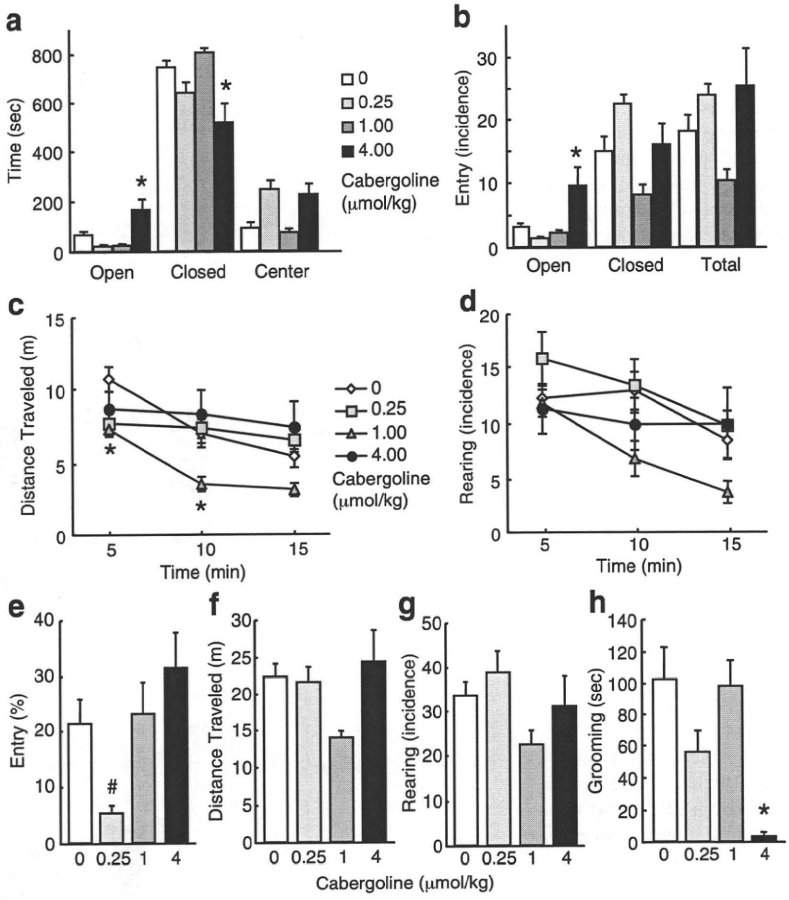

Fig. 3 Effects of single administration of cabergoline observed in EPT **a** time spent in open arm, closed arm, and center in cabergoline- and vehicle-treated rats; **b** the number of entries into arms; **c** time course curves of distance; **d** time course curves of rearing; **e** the percentage of entries into open arms per total entries; **f** total distance traveled; **g** total number of rearing; and **h** the time of grooming behavior. *Symbols/columns* and *bars* represent mean±SEM. n=7–10 for each group. *p<0.05, #p<0.1 vs. vehicle

number of rearing (t=0.47, p=0.65; Fig. 6d, g) and percentage of entries into open arms per total number (t=0.83, p=0.42; Fig. 6e). There was a trend for decreased time of grooming by the treatment (t=−1.89, p=0.079; Fig. 6h).

Effects of chronic cabergoline administration on BDNF protein expression and its related signaling

As shown in Fig. 7a, b, the chronic cabergoline treatment induced a 1.6-fold increase in the expression level of BDNF (t=4.6, p=0.002) in the homogenates from hippocampus. In contrast, no significant differences were detected in the expression levels of either BDNF receptor, i.e., p75 (t=1.4, p=0.21) or TrkB (t=1.3, p=0.22). When we examined downstream signals of TrkB, marked activation (phosphorylation) of ERK1 (pERK1, t=3.5, p=0.008; Fig. 7c, d) was observed (1.9 times higher than vehicle control). The same increasing tendency was caused by chronic cabergoline in the pERK2 level (t=2.0, p=0.074). On the other hand, no significant difference was observed in the pAkt that is another downstream signal of TrkB (t=0.8, p=0.44). In our

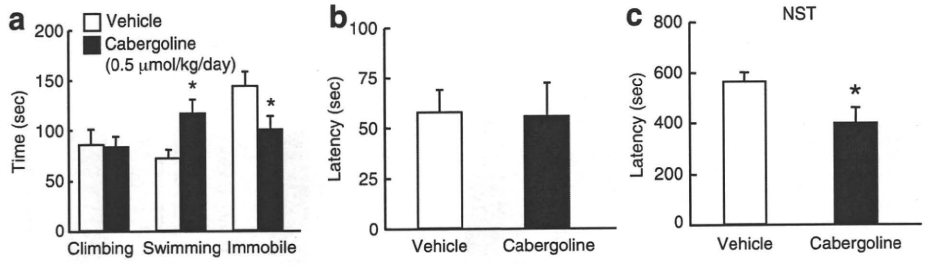

Fig. 4 Effects of chronic (14 days) administration of cabergoline (0.5 μmol/kg BW) observed in FST and NST **a** time in climbing, swimming, and immobility in cabergoline- and vehicle-treated rats in FST; **b** latency of the immobile behavior in FST; **c** latency of feeding in the NST. *Columns* and *bars* represent mean±SEM. n=8 for each group. *p<0.05 vs. vehicle

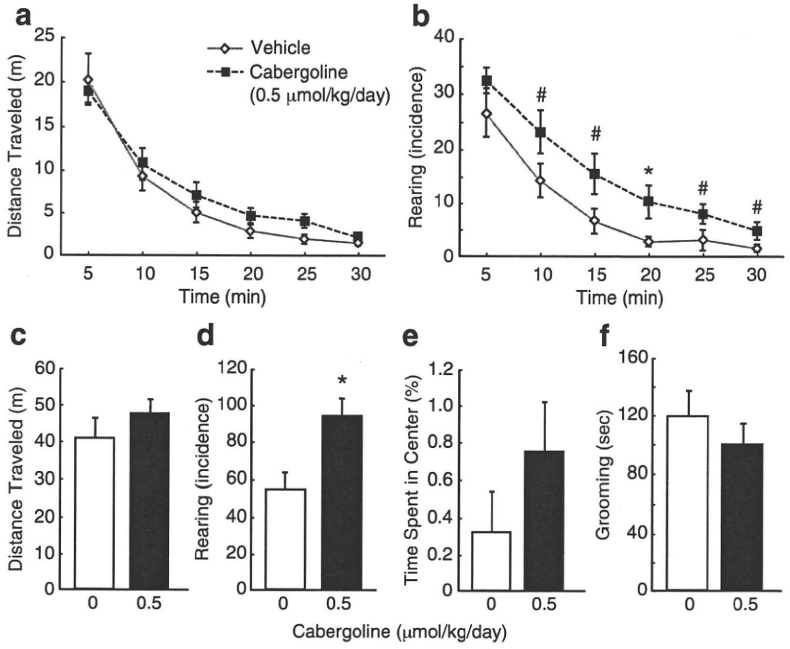

Fig. 5 Effects of chronic administration of cabergoline observed in OFT **a** time course curve of distance traveled, **b** time course curve of the number of rearing, **c** total distance traveled, **d** total number of rearing, **e** time spent in center, and **f** time of grooming. *Symbols/columns* and *bars* represent mean±SEM. $n=8$ for each group. *$p<0.05$, #$p<0.1$ vs. vehicle

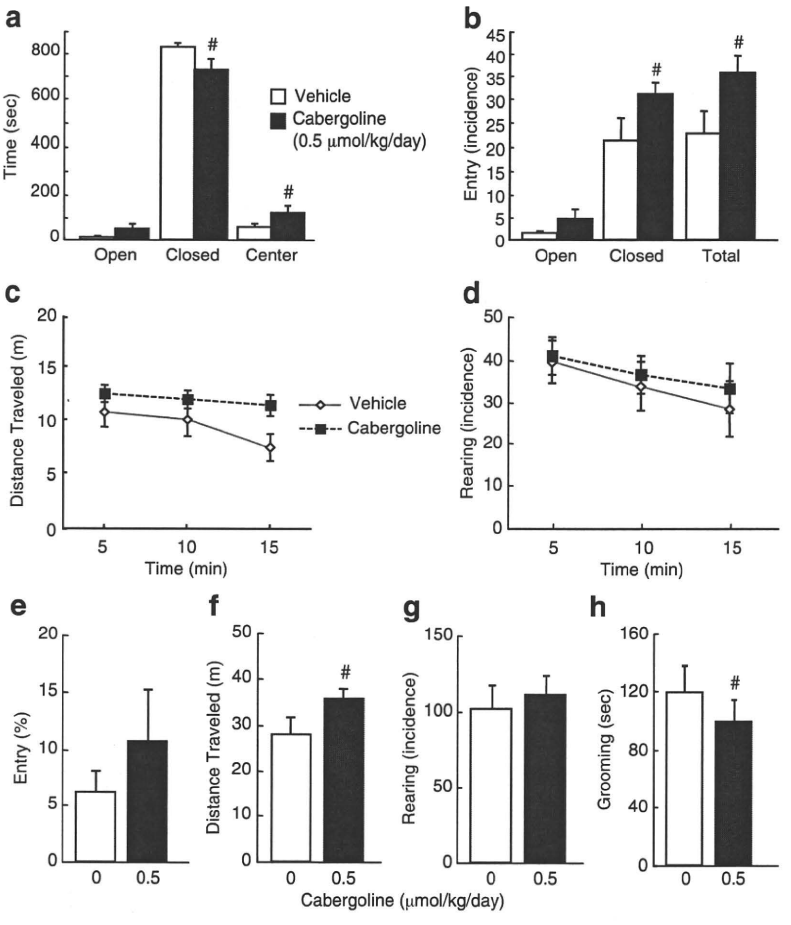

Fig. 6 Effects of chronic administration of cabergoline observed in EPT **a** time spent in each arm, **b** number of entries into each arm, **c** time course of distance traveled, **d** time course of the number of rearing, **e** the percentage of entries into open arms per total number of entries, **f** total distance traveled, **g** total number of rearing, and **h** time of grooming. *Symbols/columns* and *bars* represent mean±SEM. $n=8$ for each group. *$p<0.05$, #$p<0.1$ vs. vehicle

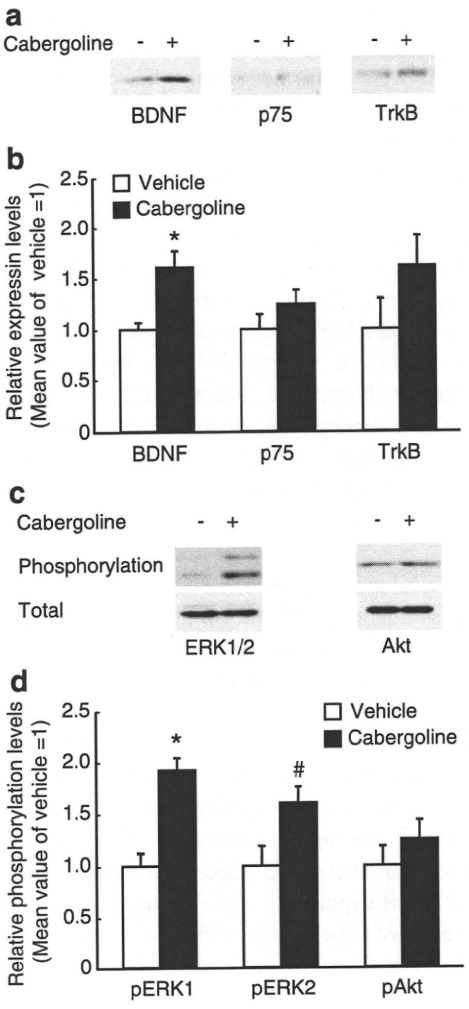

Fig. 7 Effects of chronic administration of cabergoline on the expression and activation of BDNF-related signals **a** representative blotting of BDNF (*left*), p75 (*center*), and TrkB (*right*); **b** densitometically quantified values of BDNF and its receptors; **c** representative blotting of pERK1/2 (*left*) and pAkt (*right*); **d** densitometically quantified values of BDNF-related signals. *Columns* and *bars* represent mean±SEM. $n=6$ for each group. $*p<0.05$, $\#p<0.1$ vs. vehicle

system, total expression of ERK1/2 or Akt was not altered by chronic cabergoline (Fig. 7c).

Discussion

The present study demonstrated that cabergoline reduced immobility in both acute and chronic treatment regimen. In NST, the delay in the first feeding was shortened by the repeated administration of this substance. Single treatment with low-dose cabergoline decreased distance traveled in OFT/EPT, while the high-dose treatment increased the time spent as well as the number of entries into open arms in EPT. Chronic treatment with cabergoline increased the number of rearing in OFT and tended to increase the distance traveled in EPT. In addition, our system showed that the 2-week treatment with cabergoline increased the protein level of BDNF and activation of ERK1 in hippocampus.

Our results corroborated the previous reports about antidepressant-like effect of dopamine agonists in the FST (Millan et al. 2004a; Basso et al. 2005) that has been considered as a useful test to measure the antidepressant-like properties of substances (Porsolt et al. 1977). Basso et al. (2005) demonstrated that agonists for D_2/D_3 dopamine receptors (quinpirole and PD128907), but not D_4 receptor (PD168077 and CP226269), reduced the immobility and induced the climbing behavior in the FST. Cabergoline has relatively high affinities to human D_2/D_3 dopamine receptors (Millan et al. 2002); therefore, its agonistic effect to these receptors may be attributable to the behavioral changes in the FST. In addition, chronic treatment with cabergoline reduced the latency of feeding in the novel environment that has been reported to be sensitive by the chronic antidepressant administration (Bodnoff et al. 1989). To our knowledge, this is the first evidence that dopamine agonist stimulates feeding behavior in the novel environment, although further studies are needed to elucidate its mechanism of action. Nevertheless, these results support the possibility that cabergoline has an antidepressant-like property.

Wistar-Kyoto rats displayed 2.5 times longer length of immobility in comparison with Wistar rats. In Wistar-Kyoto rats, the length of climbing behavior was increased, and that of immobility tended to be attenuated by cabergoline, but not by fluvoxamine administration. These results are consistent with previous findings that fluoxetine (SSRI) as well as 8-OH-DPAT (5-HT_{1A} agonist) were ineffective in Wistar-Kyoto rats (López-Rubalcava and Lucki 2000). Tejani-Butt et al. (2003) also showed that chronic treatment with nomifensine (a norepinephrine and dopamine reuptake inhibitor), but not with paroxetine (SSRI), reduced the immobility in FST. Interestingly, the expression level of D_2 receptor in Wistar-Kyoto rats is higher than Wistar in the ventral tegmental area where this receptor functions as an autoreceptor (Yaroslavsky et al. 2006). Swimming stress has been shown to enhance the metabolism of dopamine and 5-HT in the prefrontal cortex of Wistar-Kyoto, but not in Wistar rats (De La Garza and Mahoney 2004). These reports together with our study support the possibility that aberrant dopamine transmission and/or metabolism is involved in hyper-responsiveness to stress in Wistar-Kyoto rats and that dopamine agonists including cabergoline are effective to this strain which is refractory to SSRI.

Cabergoline at low doses demonstrated an anxiogenic effect slightly, but not significantly, while it showed a

striking anxiolytic-like effect at 4 μmol/kg BW. Other dopamine agonists such as ropinirole (Rogers et al. 2000), 7-OH-DPAT (Rogóz et al. 2004), and S32504 (Millan et al. 2004a) have been reported to demonstrate a dose-responsive anxiolytic-like property, suggesting that anxiolytic-like effect may somewhat differ between cabergoline and the other dopamine agonists. This may be attributable in part to the differential affinities for D_2 and D_3 receptors; cabergoline has similar affinities for the two receptors, while the other dopamine agonists have relatively higher affinity for D_3 over D_2 receptors (Millan et al. 2002, 2004a). Especially, preferential D_3 receptor agonist 7-OH-DPAT exerts its anxiolytic-like effect at lower doses than its optimum dose needed for antidepressant-like action (Rogóz et al. 2004). Therefore, it is possible that the action through D_3 receptor may explain the differential anxiolytic-like effects between dopamine agonists. Importantly, the chronic treatment negated the difference in the optimum doses for antidepressant and anxiolytic-like effects of cabergoline. Changes in the action of dopamine agonist after repeated administration were reported previously. The acute stimulation of D_2-like receptor by quinpirole has a dose-dependent bi-phasic effect that reduces the locomotor activity in lower doses (Eilam and Szechtman 1989). Another D_2-like agonist S32504 was also found to inhibit activities in a novel environment by a single administration (Millan et al. 2004b). On the other hand, repeated quinpirole treatment increases locomotor activity in a time-dependent manner (Szechtman et al. 1994; Rowlett et al. 1995). Our results are consistent with these observations. The time-dependent changes in the behavior might be mediated by down-regulation of D_2 autoreceptor by repeated treatment with its agonists as discussed by Szechtman et al. (1994). Another possibility is synaptic neuroadaptation after dopamine transmission. Koeltzow et al. (2003) reported similarities in dopamine outflow in the nucleus accumbens between acute and chronic quinpirole administrations, and suggested the lack of necessity of subsensitized D_2 autoreceptor for behavioral activation after the chronic treatment. From the results of the current study, it is possible that this adaptation by chronic dopamine agonist treatment is mediated by up-regulated BDNF, an important molecule for the modulation of synaptic transmission as discussed below.

We found that repeated administration of cabergoline induced the up-regulation of BDNF and activation of ERK1 in the hippocampus. Many studies found the relationship between ERK, a downstream signaling via TrkB stimulated by BDNF, and the pathophysiology of depression. Dwivedi et al. (2001) reported the inactivation and reduced expression of ERK1/2 in the prefrontal cortex and hippocampus of postmortem brains of individuals with depression. Such a reduced phosphorylation of ERK1/2 was observed in the prefrontal cortex and hippocampus of depression-model rats (Feng et al. 2003; Qi et al. 2006). In contrast, inverse phenomena were confirmed in rats after chronic treatments with fluoxetine (Qi et al. 2008), imipramine (Fumagalli et al. 2005), and mood stabilizers (Einat et al. 2003). BDNF exerts its effects on cell survival (Hetman et al. 1999) and synaptic transmission (Ying et al. 2002) via ERK signaling. Recently, we found that BDNF enhances synaptic maturation via TrkB/ERK signaling and triggers release of glutamate, an excitatory neurotransmitter, in vitro (Kumamaru et al. 2008; Numakawa et al. 2009). Furthermore, we previously showed that antidepressants (imipramine and fluvoxiamine) reinforced the BDNF-triggered glutamate release (Yagasaki et al. 2006). These findings together with the present results suggest that BDNF and ERK signaling are involved in the molecular basis of the action of antidepressants, including cabergoline, which can be attributable to synaptomodulation by BDNF.

In conclusion, the present study suggests that cabergoline has antidepressant- and anxiolytic-like properties in both acute and chronic treatment regimen. Because BDNF signaling is involved in the action of antidepressants and modulates synaptic maturation and transmission, these actions of cabergoline may be mediated by the increased BDNF/ERK signaling. In addition, we found that cabergoline showed the trend for antidepressant-like action in an innate depression-model-rat strain of Wistar-Kyoto that is known to be refractory to SSRI treatments. These results suggest that cabergoline is a promising drug candidate for the treatment of patients with depression who are refractory to SSRI.

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Relationships between psychological distress, coping styles, and HPA axis reactivity in healthy adults

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ABSTRACT

Psychological distress and coping styles have been suggested to relate to altered function in the hypothalamic-pituitary-adrenal (HPA) axis, although there remains much to be understood about their relationships. High and low cortisol levels (or reactivity) both represent HPA axis dysfunction, with accumulated evidence suggesting that they are linked to different types of psychopathology. The dexamethasone (DEX)/corticotropin-releasing hormone (CRH) test has been extensively used to identify HPA axis abnormalities in various psychiatric conditions including mood disorders; however, the possible associations of psychological distress and coping styles with HPA axis function have not been well documented using this test. Here, we examined the relationships of HPA axis reactivity as measured by the DEX/CRH test with subjectively perceived psychological distress and coping styles, both of which were assessed with self-report questionnaires, in 121 healthy volunteers. Subjects were divided into three groups by the cortisol suppression pattern, namely the incomplete-suppressors (DST-Cortisol $\geq$ 5 μg/dL or DEX/CRH-Cortisol $\geq$ 5 μg/dL), moderate-suppressors (DST-Cortisol $<$ 5 μg/dL and 1 μg/dL $\leq$ DEX/CRH -Cortisol $<$ 5 μg/dL), and enhanced-suppressors (DST-Cortisol $<$ 5 μg/dL and DEX/CRH-Cortisol $<$ 1 μg/dL). The enhanced-suppressors showed significantly higher scores in obsessive-compulsive, interpersonal sensitivity and anxiety symptoms and significantly more frequent use of avoidant coping strategy, compared to the other two groups. These results point to the important role of enhanced suppression of cortisol, or blunted cortisol reactivity, in non-clinical psychopathology such as avoidant coping strategy and greater psychological distress.

1. Introduction

A wide variety of stress is associated with alteration in the hypothalamic-pituitary-adrenal (HPA) axis function. Studies looking at cortisol as the main output substance of the HPA axis have thus been critical to advancing our understanding of psychobiological underpinnings of various stress-related conditions (de Kloet et al., 2005; Heim et al., 2000). For instance, perceived stress in everyday life (Pruessner et al., 1999), stressful situations such as academic examinations and seafaring (Droogleever Fortuyn et al., 2004; Liberzon et al., 2008), self-reported symptoms (Van den Bergh et al., 2008), psychological coping styles (Nicolson, 1992; O'Donnell et al., 2008), rejection sensitivity (Tops et al., 2008), sleep status (Backhaus et al., 2004; Lasikiewicz et al., 2008; Wright et al., 2007) and personality profile (Tyrka et al., 2007) have been reported to be associated with alteration in cortisol levels. These studies in healthy subjects have investigated HPA axis function using several different cortisol measures including diurnal cortisol profiles, cortisol awakening response, and cortisol reactivity to psychosocial challenge tests such as Trier Social Stress Test (Kirschbaum et al., 1993).

On the other hand, HPA axis function in clinical populations, particularly in patients with major depression, has been investigated with pharmacological challenge tests including dexamethasone (DEX) suppression test (DST, Carroll et al., 1976) and DEX/corticotropin-releasing hormone (CRH) test (Heuser et al., 1994a;

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Holsboer et al., 1987). The DEX/CRH test is an integrated challenge test for HPA axis function that combines DEX-pretreatment with CRH administration on the following day; thus, it is essentially a DST followed by CRH challenge. The merit of this combined test is that at the moment of CRH infusion the HPA axis is downregulated due to negative feedback induced by the DEX. In the DEX/CRH test a relatively high dose (i.e., 1.5 mg) of DEX is usually used, whereas DST studies, in particular those which examine the HPA function of post-traumatic stress disorder (PTSD), have used a lower dose (i.e., 0.5 mg or 1 mg) of DEX (e.g., Grossman et al., 2003; Yehuda et al., 2004). Sensitivity of the DEX/CRH test in depressed patients has been shown to be high in prior studies including ours (Heuser et al., 1994a; Kunugi et al., 2004, 2006; Watson et al., 2006b). Moreover, this test has revealed altered HPA axis function in those individuals with specific characteristics: dampened cortisol reactivity in healthy adults reporting childhood emotional abuse (Carpenter et al., 2009), increased cortisol responses in healthy adults reporting childhood parental loss with the exception of attenuated cortisol responses in those with parental desertion and low levels of care (Tyrka et al., 2008b), increased cortisol responses in healthy adults with certain personality traits (Tyrka et al., 2006, 2008a), and attenuated cortisol responses in depressed women on job-stress-related longterm sickleave (Rydmark et al., 2006; Wahlberg et al., 2009). On the other hand, the possible associations of more commonly presented psychopathology such as perceived distress in everyday life and coping styles with HPA axis function have not been well documented using the DEX/CRH test. However, these psychological measures are suggested to relate to altered cortisol level (Heim et al., 2000, 2002; Nicolson, 1992; O'Donnell et al., 2008; Pruessner et al., 1999; Van den Bergh et al., 2008). For instance, severity of daily hassles in the past month was negatively related to cortisol concentrations (Heim et al., 2002). Perceived stress was positively, and burnout was negatively, associated with cortisol levels after DEX administration (Pruessner et al., 1999). Passive coping is suggested to relate to hypocortisolism (Heim et al., 2000). Healthy adults scoring high in either problem engagement or seeking social support showed lower cortisol levels (O'Donnell et al., 2008). Given these findings, it would be of interest to examine HPA axis function in relation to psychopathology at a non-clinical level such as psychological distress and coping styles by using the DEX/CRH test.

Various kinds of psychiatric disorders have been shown to be associated with HPA axis hyperactivity as reflected by the high cortisol levels and impaired negative feedback inhibition due to an impaired corticosteroid receptor function (Holsboer, 2000). On the other hand, a number of psychoneuroendocrinological studies have demonstrated that a variety of conditions are associated with hypocortisolism, including low basal cortisol levels, enhanced sensitivity to the negative feedback, and blunted reactivity of provoked cortisol. Examples of psychiatric conditions characterized by hypocortisolism include PTSD, chronic fatigue syndrome, fibromyalgia and atypical depression (Fries et al., 2005; Heim et al., 2000). Together, while both of these two extremes of cortisol activity can represent HPA axis dysfunction, they are likely to be linked to different types of psychopathology. Concerning hypocortisolism, there remains much to be clarified as to its natural course and meaning. Although hypocortisolism is considered to represent the result of prolonged stress exposure (Fries et al., 2005; Heim et al., 2000; Ising et al., 2005), a condition so-called "allostasis" (McEwen, 2003), there also exists some evidence suggesting that this state could be a preexisting vulnerability to stress-related disorders (Delahanty et al., 2000; Wahlberg et al., 2009; Yehuda et al., 2000).

Arginine vasopressin (AVP), in addition to CRH, is an HPA axis secretagogue. AVP produced in parvocellular neurons of hypothalamic paraventricular nucleus (PVN) and secreted into pituitary portal vein system plays an important role in stress response (Herman, 1995; Romero and Sapolsky, 1996). It is reported that, in chronic stress paradigms, the expression of AVP in parvocellular neurons increases and pituitary V1b receptor, through which AVP stimulates the ACTH secretion, up-regulates (Aguilera et al., 1994; Aguilera and Rabadan-Diehl, 2000). There also exist clinical studies that support this notion. For example, de Kloet et al. (2008) have recently reported elevated plasma AVP levels in veterans with PTSD. Watson et al. (2006a) measured plasma AVP levels after pre-treatment of DEX in patients with chronic depression and those with bipolar disorder, and found significantly higher post-DEX AVP levels in the patient groups than in healthy controls, suggesting that post-DEX AVP levels could be more sensitive than baseline AVP levels in detecting HPA axis abnormalities. These findings raise the possibility that the post-DEX AVP measure may help understand whether the hypocortisolism, if present, is a result of chronic HPA axis overactivity or a preexisting vulnerability factor for psychopathology.

In this context, the present study sought to examine the relationships between subjectively perceived psychological distress, psychological coping styles and the cortisol suppression pattern to the DEX/CRH test in non-clinical volunteers. We also examined the relationships of these psychological measures with the post-DEX AVP level to see whether the possible low cortisol levels would reflect allostatic shift or preexisting vulnerability. The study hypothesis was that the higher cortisol levels (or less suppression of cortisol) and/or lower cortisol levels (or more suppression of cortisol) would be related to greater distress and a unique pattern of coping strategies. If the low cortisol, together with elevation of AVP, is related to these psychological measures, it would indicate allostatic shift; while if the low cortisol, together with no elevation of AVP, is related to such psychological measures, it may indicate preexisting vulnerability.

2. Materials and methods

2.1. Participants

From February 2006 to December 2008, 121 healthy volunteers (age range: 20–70; male, 28, female, 93) were recruited from the community, through advertisements in free local information magazines which contained a wide variety of information including healthcare-related information and by our website announcement. Participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview (MINI, Otsubo et al., 2005; Sheehan et al., 1998) by research psychiatrists (H.H., Y.O., T.T. and H.K.), and only those who demonstrated no current Axis I psychiatric disorders, including PTSD, were enrolled in this study. In addition, those who demonstrated one or more of the following conditions during a non-structured interview by an experienced psychiatrist were excluded from this study: past or current contact to psychiatric services, taking psychotropic drugs or had a history of regular use of psychotropics, and the other obvious self-reported signs of past primary psychotic and mood disorders as well as PTSD. Additional exclusion criteria were as follows: having a prior medical history of central nervous system disease or severe head injury, having major systemic medical illnesses, having a history of substance dependence or abuse, or taking corticosteroids or antihypertensive medication. No subjects reported that they were on oral contraceptives or estrogen replacement therapies. The present experiments on our subjects were conducted in accordance with the Declaration of Helsinki. After the nature of the study procedures had been fully explained, written informed consent was obtained

from all subjects. The study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. DEX/CRH test procedure and presentation for neuroendocrine data

The DEX/CRH test was administered to all subjects by a single examiner (H.H.) according to a protocol proposed in a previous report (Kunugi et al., 2006). First, they took 1.5 mg of DEX (Banyu Pharmaceutical Corporation, Tokyo, Japan) orally at 2300 h. On the next day, they attended our laboratory and sat on a comfortable couch in a calm room. A vein was cannulated at 1430 h to collect blood at 1500 and 1600 h via an intravenous catheter. Human CRH (100 μg) (hCRH 'Mitsubishi', Mitsubishi Pharma Corporation, Tokyo, Japan) was administered intravenously at 1500 h, immediately after the first blood collection. Subjects fasted and rested semi-supine throughout the testing. Blood samples were immediately centrifuged and stored at −20 °C. Plasma concentrations of cortisol and AVP were measured by radioimmunoassay at SRL Corporation (Tokyo, Japan). The detection limits for cortisol and AVP were 1.0 μg/dL and 0.2 pg/mL, respectively (SRL Corporation, Tokyo, Japan). Cortisol and AVP values under the detection limits were treated as 0 μg/dL and 0 pg/mL, respectively. For cortisol, the intra-assay coefficients of variation at 2.37 μg/dL, 13.02 μg/dL, and 36.73 μg/dL were 6.90%, 4.94%, and 5.78%, respectively. The inter-assay coefficients of variation at 2.55 μg/dL, 13.04 μg/dL, and 34.17 μg/dL were 8.91%, 6.03%, and 6.44%, respectively. For AVP, the intra-assay coefficients of variation at 0.97 pg/mL, 1.64 pg/mL, and 2.88 pg/mL were 1.7%, 7.2%, and 3.5%, respectively. The inter-assay coefficients of variation at 0.94 pg/mL, 1.59 pg/mL, and 2.88 pg/mL were 3.9%, 10.3%, and 6.9%, respectively (SRL Corporation, Tokyo, Japan). Outcome measures of this neuroendocrine test were the DST-Cortisol (i.e., the concentration of cortisol [μg/dl] at 1500 h), DEX/CRH-Cortisol (i.e., the concentration of cortisol at 1600 h) and DST-AVP (i.e., the concentration of AVP [pg/ml] at 1500 h). To further dissect the extent to which the subject's HPA axis responded to the CRH challenge, the magnitude of change from DST-Cortisol to DEX/CRH-Cortisol, namely ΔCortisol, was calculated for each subject. For DST-AVP, data were available for only 106 of the total 121 subjects. This reduction of subjects was because we started to collect the AVP data on May 2006, which was about 3 months after the study initiation.

A cut-off criterion for suppression status was considered as follows; 'Incomplete-suppressors' were defined *a priori* to be individuals where either or both of DST- and DEX/CRH-Cortisols were equal to or more than 5 μg/dL. This cut-off value was based on our previous studies (Kunugi et al., 2004, 2006), where the cortisol value of 5 μg/dL was shown to sensitively distinguish depressed patients from healthy controls. Based on these reports of ours, recent studies (Ising et al., 2007; Schüle et al., 2009) also used the same cut-off value of cortisol. Given these findings, in the present study we assumed that the cortisol value of 5 μg/dL would be also useful in detecting those participants whose negative feedback of cortisol was "incomplete". On the other hand, 'Enhanced-suppressors' were defined as those individuals whose DST-Cortisol was less than 5 μg/dL and DEX/CRH-Cortisol was less than 1 μg/dL, because this DEX/CRH-Cortisol value corresponded to its detection limit and can therefore be regarded as an extremely low cortisol level. The remaining individuals were considered to be 'Moderate-suppressors'. We would like to note that the 'Incomplete-suppressors' were the sum total of the 'Intermediate suppressors' and 'Non-suppressors' as defined in our previous studies (Kunugi et al., 2004, 2006). This slight modification on the grouping criterion was because it was expected that very few subjects would fall into the 'Non-suppressors' group since the present study included only healthy subjects.

2.3. Psychological assessment

To assess subjectively perceived psychological distress and psychological coping styles, the following two self-report questionnaires were distributed to the participants.

2.3.1. The hopkins symptom checklist (HSCL)

Subjectively perceived psychological distress during one week preceding the neuroendocrine test was assessed via the Hopkins Symptom Checklist (HSCL, Derogatis et al., 1974), a self-report questionnaire consisting of 58 (or 54) items which are scored on 5 underlying symptom dimensions, i.e., somatization, obsessive-compulsive, interpersonal sensitivity, anxiety, and depression symptoms. In the present study, a validated Japanese version of the HSCL (Nakano, 2005) comprising 54 items was used. In this questionnaire, subjects were instructed to rate each item based on the distress perceived during the previous week, using a four-point scale of frequency, with "not-at-all" being scored 1, "occasionally", 2, "sometimes", 3, and "frequently", 4. All of the 121 participants answered this questionnaire.

2.3.2. The ways of coping checklist (WCCL)

Psychological coping can be defined as the thoughts and behaviors used to manage the internal and external demands of situations that are appraised as stressful (Folkman and Moskowitz, 2004). Coping styles of the participants were assessed using the Japanese version of the Ways of Coping Checklist (WCCL) (Folkman and Lazarus, 1985; Nakano, 1991), a self-report questionnaire consisting of 47 items which measure each participant's preferred coping styles using a four-point scale of frequency, with "not used" being scored 0, "not frequently used", 1, "sometimes used", 2, and "regularly used", 3. The 47 items were grouped into 6 coping strategies, namely planful problem-solving, positive reappraisal, seeking social support, self-blame, wishful thinking, and escape-avoidance (Nakano, 1991), thus measuring both problem-focused and emotion-focused coping strategies. The WCCL data were obtained from 102 of the total 121 participants. This reduction of participants was because we started to collect the WCCL data on June 2006.

2.4. Statistical analysis

Averages are reported as means ± SD (standard deviation). Categorical variables were compared using the χ^2 test. Mann–Whitney *U*-test was used to compare hormonal measures between two groups. The analysis of variance (ANOVA) or Kruskal–Wallis test was used to examine differences between three groups. Pearson's r was used to examine correlations among psychological measures, while Spearman's ρ was used to examine correlations among hormonal data or between hormonal data and psychological measures. To examine the difference between DST- and DEX/CRH-Cortisols, Wilcoxon signed rank test was performed. The analysis of covariance (ANCOVA), controlling for confounding variables, was performed to compare the scores of questionnaires between the three participant groups. Since age and sex have been shown to significantly influence the cortisol levels (e.g., Heuser et al., 1994b; Kunugi et al., 2006; Kunzel et al., 2003), these variables were considered as confounders regardless of the present data. Statistical significance was set at two-tailed $p < 0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

Table 1
Correlations between coping styles and psychological distress (Pearson's r).

	Somatization	Obsessive-compulsive	Interpersonal sensitivity	Anxiety	Depression
Problem-solving	−0.10	−0.22*	−0.24*	−0.17	−0.25*
Positive reappraisal	−0.08	−0.16	−0.26**	−0.20*	−0.32**
Social support	0.11	0.14	0.14	0.16	−0.03
Self-blame	0.29**	0.47***	0.52***	0.49***	0.48***
Wishful thinking	0.22*	0.42***	0.37***	0.40***	0.31**
Escape-avoidance	0.08	0.30**	0.33***	0.30**	0.20*

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3. Results

3.1. Demographic characteristics of the subjects

The numbers of incomplete-suppressors, moderate-suppressors, and enhanced-suppressors were 55, 54, and 12, respectively, indicating that the enhanced-suppressors corresponded to approximately the bottom 10% of total subjects for cortisol levels. The mean ages of incomplete-suppressors, moderate-suppressors, and enhanced-suppressors were 46.8 ± 14.3, 42.7 ± 15.1, and 44.4 ± 14.7, respectively. These three suppression groups did not significantly differ in age [$F(2,118) = 1.49$, $p = 0.23$]. Male/female ratios of incomplete-suppressors, moderate-suppressors, and enhanced-suppressors were 6/49, 15/39, and 7/5, respectively. There was a significant difference in sex distribution [$\chi^2(2) = 13.6$, $p = 0.001$]; males demonstrated significantly greater suppression than females.

3.2. Correlations between coping styles and psychological distress

Table 1 shows the correlations between coping styles assessed with the WCCL and psychological distress assessed with the HSCL. Significant negative correlations were seen between problem-focused coping strategies (i.e., problem-solving and positive reappraisal) and greater psychological symptoms including interpersonal sensitivity and depression. In contrast, significant positive correlations were observed between emotion-focused coping strategies (i.e., self-blame, wishful thinking and escape-avoidance) and most of the symptom dimensions. Social support was not significantly related to any of the symptom dimensions.

3.3. Relationships between hormonal measures

The cortisol values for the three suppressor groups on the three cortisol indices are provided in Table 2. DST-Cortisol of 64 subjects and DEX/CRH-Cortisol of 12 subjects fell under the detection limit, while DST-AVP did not fall under the detection limit in any subjects. DEX/CRH-Cortisol was significantly higher than DST-Cortisol in the whole sample, as expected ($p < 0.001$; Wilcoxon signed rank test). There was no significant correlation of DST-AVP with DST-Cortisol ($\rho = 0.07$, $p = 0.50$), DEX/CRH-Cortisol ($\rho = 0.03$, $p = 0.75$), or ΔCortisol ($\rho = 0.02$, $p = 0.83$). The three suppression groups did not significantly differ in DST-AVP [Kruskal–Wallis $\chi^2(2) = 0.14$, $p = 0.93$].

3.4. Correlations between hormonal and psychological measures

No significant correlations were seen between DST-Cortisol and any measures of the two questionnaires (all $p > 0.2$). No significant correlations were seen between DEX/CRH-Cortisol and any measures of the two questionnaires (all $p > 0.2$) except for interpersonal sensitivity ($\rho = -0.20$, $p = 0.03$) in the HSCL. Similarly, no significant correlations were observed between ΔCortisol and any measures of the two questionnaires (all $p > 0.2$) except for interpersonal sensitivity ($\rho = -0.21$, $p = 0.02$) in the HSCL. No significant correlation was found between DST-AVP and any of the outcomes of the two questionnaires (all $p > 0.1$).

3.5. Relationships between psychological measures and DEX/CRH outcomes

3.5.1. Psychological distress and DEX/CRH outcomes

Fig. 1 shows the relationships between 5 symptom dimensions of the HSCL and DEX/CRH suppression status. The ANCOVA on 5 symptoms controlling for age and sex showed significant main effects of the suppression status on obsessive-compulsive [$F(2,114) = 5.19$, $p = 0.007$], interpersonal sensitivity [$F(2,114) = 5.43$, $p = 0.006$], and anxiety [$F(2,114) = 5.86$, $p = 0.004$], symptoms. Post-hoc analyses with Bonferroni correction revealed that the enhanced-suppressors, compared to the other two groups or to moderate-suppressors alone, had significantly greater scores on these three symptom dimensions, while no significant differences were seen between incomplete- and moderate-suppressors (Fig. 1).

However, a considerable portion of the subjects fell into the incomplete-suppressors and thus we considered that this group would not necessarily represent those individuals whose cortisol levels were abnormally high. Therefore, to confirm the results obtained by the main analysis, the same ANCOVA was repeated with another grouping criterion as follows: 'incomplete-suppressors' to be individuals where either or both of DST- and DEX/CRH-

Table 2
Plasma cortisol concentrations (mean ± SD (range)) for the three subject groups, based on the suppression pattern.

	Incomplete-suppressors ($n = 55$)[d]	Moderate-suppressors ($n = 54$)[e]	Enhanced-suppressors ($n = 12$)[f]
DST-Cortisol[a]	1.4 ± 1.5 (0 ~ 5.8)	0.4 ± 0.7 (0 ~ 1.9)	0.1 ± 0.3 (0 ~ 1.1)
DEX/CRH-Cortisol[b]	10.0 ± 4.6 (5.0 ~ 25.1)	2.5 ± 1.1 (1.1 ~ 4.9)	0 ± 0 (0 ~ 0)
ΔCortisol[c]	8.6 ± 4.4 (2.3 ~ 20.2)	2.1 ± 1.3 (−0.3 ~ 4.8)	−0.1 ± 0.3(−1.1 ~ 0)

[a] The concentration of cortisol [μg/dl] at 1500 h (i.e., immediately before the CRH challenge).
[b] The concentration of cortisol [μg/dl] at 1600 h (i.e., 1 h after the CRH challenge).
[c] Defined as "DEX/CRH-Cortisol minus DST-Cortisol".
[d] Defined as "DST-Cortisol ≥ 5 or DEX/CRH-Cortisol ≥ 5".
[e] Defined as "DST-Cortisol < 5 and 1 ≤ DEX/CRH -Cortisol < 5".
[f] Defined as "DST-Cortisol < 5 and DEX/CRH-Cortisol < 1".

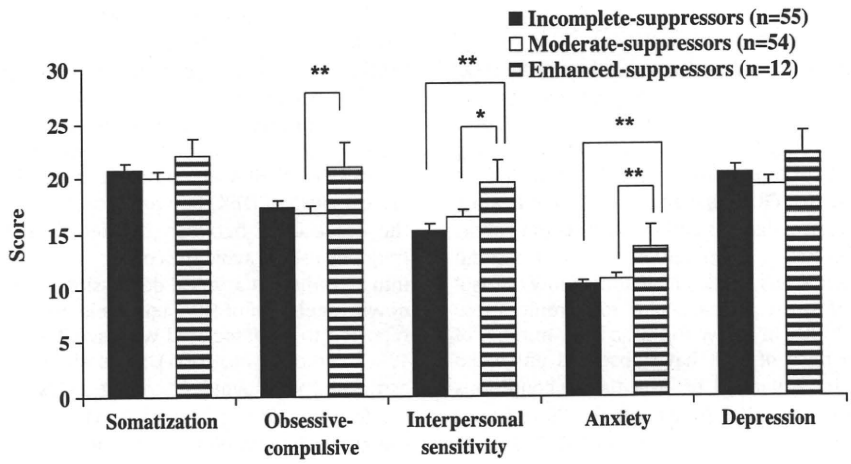

Fig. 1. Comparisons of scores on the 5 dimensions of the Hopkins Symptom Checklist (HSCL) between the three suppression groups. Black, white, and borderline bars are incomplete-suppressors (defined as "DST-Cortisol ≥5 or DEX/CRH-Cortisol ≥5"; $n = 55$), moderate-suppressors (defined as "DST-Cortisol <5 and 1 ≤ DEX/CRH -Cortisol <5"; $n = 54$), and enhanced-suppressors (defined as "DST-Cortisol <5 and DEX/CRH-Cortisol <1"; $n = 12$), respectively. *$p < 0.05$; **$p < 0.01$. Error bars represent standard errors of the mean.

Cortisols were equal to or more than 13 μg/dL, 'enhanced-suppressors' to be those whose DST-Cortisol was less than 13 μg/dL and DEX/CRH-Cortisol was less than 1 μg/dL, and 'moderate-suppressors' to be the remaining individuals. The reason why we here used the cortisol level of 13 μg/dL, instead of the original 5 μg/dL, as the cut-off value for the 'incomplete-suppressors' was that this value corresponded to approximately the top 10% of total subjects for cortisol levels. This 10% derived from the fact that the cortisol value of "enhanced-suppressors" corresponded to approximately the bottom 10% of total subjects. Using this new grouping, additional ANCOVA on the 5 symptoms controlling for age and sex was performed, again showing significant main effects of the suppression status on obsessive-compulsive [$F(2,114) = 4.63$, $p = 0.012$], interpersonal sensitivity [$F(2,114) = 5.50$, $p = 0.005$] and anxiety [$F(2,114) = 5.81$, $p = 0.004$] symptoms. Post-hoc analyses with Bonferroni correction revealed that the enhanced-suppressors, compared to the other two groups or to moderate-suppressors alone, scored significantly higher on these three symptom dimensions, while no significant differences were seen between incomplete- and moderate-suppressors (data not shown).

3.5.2. Coping styles and DEX/CRH outcomes

The relations between the 6 different coping styles of WCCL and suppression status are provided in Fig. 2. The ANCOVA on the 6 coping subscales controlling for age and sex showed a significant main effect of the suppression status on escape-avoidance [$F(2,95) = 5.26$, $p = 0.007$]. Post-hoc analyses with Bonferroni correction revealed that the enhanced-suppressors, compared to the other two groups, had significantly greater scores on this coping strategy, while no significant differences were found between incomplete- and moderate-suppressors (Fig. 2).

The additional ANCOVA with the other grouping criterion of suppression status on the 6 coping subscales showed significant main effects of the suppression status on wishful thinking [$F(2,95) = 3.31$, $p = 0.041$] and escape-avoidance [$F(2,95) = 5.56$, $p = 0.005$]. Post-hoc analyses with Bonferroni correction revealed that the enhanced-suppressors, compared to the other two groups or to incomplete-suppressors alone, scored significantly higher on these two coping strategies, while no significant differences were seen between incomplete- and moderate-suppressors (data not shown).

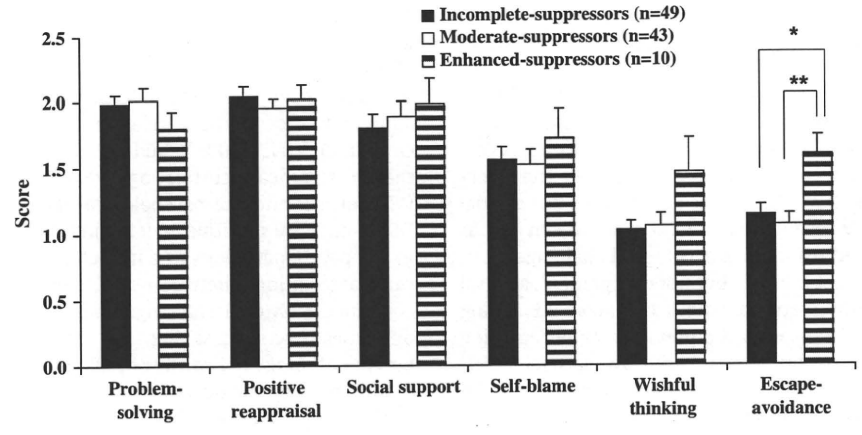

Fig. 2. Comparisons of scores on the 6 subscales of the Ways of Coping Checklist (WCCL) between the three suppression groups. Black, white, and borderline bars are incomplete-suppressors (defined as "DST-Cortisol ≥5 or DEX/CRH-Cortisol ≥5"; $n = 49$), moderate-suppressors (defined as "DST-Cortisol <5 and 1 ≤ DEX/CRH-Cortisol <5"; $n = 43$), and enhanced-suppressors (defined as "DST-Cortisol <5 and DEX/CRH-Cortisol <1"; $n = 10$), respectively. *$p < 0.05$; **$p < 0.01$. Error bars represent standard errors of the mean.

4. Discussion

We examined the relationships of cortisol reactivity to the DEX/CRH test with subjectively perceived psychological distress and psychological coping styles as assessed with the self-report questionnaires in a non-clinical population. The most salient finding was that the enhanced cortisol suppression to the DEX/CRH test, or blunted cortisol response to CRH challenge, was significantly related to various psychological distress and avoidant coping style.

Besides the well-established relation between acute stress and elevated cortisol levels, numerous studies have linked low cortisol levels to various kinds of stress, in particular to chronic stress (reviewed in Heim et al., 2000). In line with this, a large number of DST studies using a low dose of DEX have observed enhanced suppression of cortisol in a variety of psychiatric conditions including PTSD (Grossman et al., 2003; Yehuda et al., 1993). To our knowledge, however, the present study is the first DEX/CRH study where the overtly defined enhanced suppression, in addition to the incomplete suppression, was examined in the context of non-clinical psychological distress and coping styles. Based on the previous literature, it was hypothesized that both incomplete and enhanced suppression of cortisol due to the negative feedback by DEX administration would be related to greater distress and/or a unique pattern of coping strategies. The significant associations of enhanced suppression with greater distress and more frequent use of avoidant coping strategy supported the hypothesis, while contrary to our prediction incomplete suppression was not significantly related to any of the psychological measures.

We observed significant negative correlations between interpersonal sensitivity in the HSCL and cortisol values, namely DEX/CRH-Cortisol and ΔCortisol. A significant relation between interpersonal sensitivity and enhanced cortisol suppression was also found. These results were in line with a recent study showing the association between higher rejection sensitivity and lower cortisol awakening responses in community women (Tops et al., 2008). In addition, the significant relationships of enhanced cortisol suppression with obsessive-compulsive and anxiety symptoms, but not depressive symptom, point to the possibility that enhanced suppression is more related to anxiety symptoms than depressive symptoms in healthy populations. Several studies investigated cortisol levels as measured by DST in patients with obsessive-compulsive disorder (OCD), and the majority of these studies found that OCD patients did not show non-suppression or their baseline cortisol levels did not differ from healthy controls (e.g., Kuloğlu et al., 2007; Lieberman et al., 1985). These previous findings, combined with the present result, might suggest that obsessive-compulsive symptoms are associated with normal cortisol suppression to DEX and subsequent blunted response to CRH challenge.

Several lines of research have documented the relationship of coping styles with psychobiological measures including cortisol levels (Frecska et al., 1988; Nicolson, 1992; O'Donnell et al., 2008). While cortisol activity has been considered to reflect the effectiveness of coping strategies (Nicolson, 1992), how differential coping styles in everyday settings relate to cortisol reactivity has not been well documented using pharmacological challenge tests. The present study found that blunted, but not exaggerated, cortisol reactivity was significantly associated with the avoidant coping style. This finding is consistent with the previous study reporting the association between passive coping and low cortisol levels (Heim et al., 2000). However, the findings to date on the association between coping styles and cortisol activity have not been unequivocal. A 1-mg DST study (Frecska et al., 1988), for example, observed an association between high post-DEX cortisol levels and denial and passivity. Furthermore, O'Donnell et al. (2008) found no significant association between the avoidant coping style and cortisol levels in healthy older adults. Instead, they found that individuals who scored higher in either problem engagement or seeking social support had lower cortisol output over the day. These inconsistent findings might be due to different instruments for the measurement of coping styles and/or to different measures for the assessment of HPA axis function (e.g., DST vs. DEX/CRH and high- vs. low-dose of DEX) between studies. Still, the present finding of the association between blunted cortisol reactivity and more frequent use of avoidant coping style might be intriguing, taking into account that atypical depression, a disorder known to relate to down-regulation of HPA axis (Gold and Chrousos, 2002), has been reported to be associated with avoidant personality (Alpert et al., 1997; Parker et al., 2005). Similarly, PTSD has been found to be associated with avoidant coping styles (Bryant and Harvey, 1995; Krause et al., 2008) as well as with low cortisol levels, including somewhat low baseline cortisol levels (Meewisse et al., 2007) and enhanced suppression of cortisol to the low dose of DEX (Grossman et al., 2003; Yehuda et al., 1993).

The associations between coping styles and psychological distress, more specifically the significant positive correlation between more frequent use of avoidant coping strategy and greater psychological distress, were in line with previous studies (Goossens et al., 2008; Spira et al., 2004). Taken together, we observed significant relationships between greater distress, avoidant coping style, and blunted cortisol response. A feasible scenario for this relation would be that psychological distress and avoidant coping style result from the failure to mobilize cortisol to adequately cope with stressors. Alternatively, persistent psychological distress and/or avoidant coping style may end up in blunted cortisol reactivity.

The potential mechanism by which the enhanced suppression was related to the psychological distress and avoidant coping style could be discussed as follows. Since the negative feedback by DEX occurs mainly at the level of the pituitary (Cole et al., 2000), the excessively suppressed cortisol response to the DEX/CRH challenge is likely to stem from high sensitivity of pituitary glucocorticoid receptor. In line with this, enhanced negative feedback inhibition at the level of the pituitary caused by the low dose of DEX (0.5 mg) is considered to underlie the enhanced suppression of cortisol and ACTH in PTSD (Yehuda et al., 2004). The present study, using a higher dose of DEX (1.5 mg), was not primarily aimed to test the association of enhanced feedback inhibition by DEX itself with psychological measures, and actually cortisol levels of a considerable portion of our subjects fell under detection limit. A DST with the higher dose of DEX pre-treatment is optimized for the detection of decreased HPA axis feedback sensitivity whereas a DST with the lower dose of DEX is more sensitive for the detection of increased HPA axis feedback sensitivity. Indeed, using a 0.5-mg DST, a number of studies have found individuals with PTSD to display enhanced suppression of cortisol relative to those without PTSD (e.g., Grossman et al., 2003; Yehuda et al., 2004). Nevertheless, the fact that no significant correlational relationships were seen between DST-Cortisol and the psychological measures might indicate that DST is not very sensitive in detecting HPA axis alteration in relation to the psychopathology at a non-clinical level. Instead, the significant associations between the enhanced suppression to the combined DEX/CRH challenge and the unfavorable psychological outcomes may suggest that HPA axis alteration in relation to the psychopathology in healthy populations would be accounted for, at least in part, by the down-regulation of CRH receptors on the level of the pituitary rather than by the enhanced feedback inhibition detectable by the DST. However, to draw any conclusions as to where in the HPA axis the alteration exists, more adequate dose of DEX for pre-treatment should be further explored.