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Table 1 Baseline feature of schizophrenic patients treated with risperidone

	New Group	Switched Group	Total	Significance*
# Patients	27	37	64	
Gender (M/F)	16/11	23/14	39/25	NS ‡ (p=0.81)
Mean age (years)	35.1±2.6 †	37.9±2.2	36.7± 13.6	NS (p=0.60)
Duration of illness (years)	6.2±2.3	13.0±2.1	10.0± 12.4	χ^2=8.5, df=1, p=0.004
Duration of hospitalization (years)	0.16±1.9	6.6±61.7	3.8±10.3	χ^2=13.2, df=1, p=0.003
Mean dose of risperidone (mg) at 8 weeks	4.0±0.38	3.9±0.34	3.9±1.8	NS (p=0.53)
Mean total score of PANSS				
Initial	90.9±4.4	70.9±3.7	79.4± 24.6	χ^2=9.7, df=1. p=0.02
8 weeks	60.4±3.6	62.6±3.1	61.7± 18.5	NS (p=0.83)

*Statistical analysis was performed using Fisher's exact test and Mann-Whitney's test. † Values are means±SD. ‡ NS, not significant.

Table 2 Diplotype configurations (combination of two haplotypes) of DRD2 (a) and HTR2A (b) in 64 schizophrenic patients

a. DRD2

	Ins-A1 (-/-)	Ins-A2 (-/+)	Del-A1 (+/-)	Del-A2 (+/+)
Ins-A1 (-/-)	2	21	0	0
Ins-A2 (-/+)		25	9	6
Del-A1 (+/-)			0	1
Del-A2 (+/+)				0

Parentheses indicate the hypothetical dopamine D2 receptor expression level by the DRD2 genotype.

b. HTR2A

	A-T	G-C	A-C	G-T
A-T	17	23	3	2
G-C		13	4	1
A-C			1	0
G-T				0

Table 3 Effects of Administrated groups (New or Switched), Baseline Psychopathology, DRD2 Haplotype of Positive and Negative Symptom Scale (PANSS) Total, and Subscale Scores

Variable	Relation to Natural Logarithm Score on PANSS, Determined by Multiple Linear Regression Analysis		
	Estimated Coefficient	SE of Estimated Coefficient	p
Total score			
Switched Group compared to New Group	-2.7053	0.8503	0.003
Baseline PANSS total score	0.0824	0.0308	0.01
DRD2 diplotype			
Ins-A2/ Ins-A2 compared to Ins-A1/ Ins-A1	1.4861	1.2744	0.25
Ins-A2/ Ins-A1 compared to Ins-A1/ Ins-A1	0.6309	1.3843	0.65
Ins-A2/ Del-A1 compared to Ins-A1/ Ins-A1	-0.0676	1.9228	0.97
Ins-A2/ Del-A2 compared to Ins-A1/ Ins-A1	4.70895	2.0192	0.02
Positive subscale			
Switched Group compared to New Group	-4.5972	2.1683	0.04
DRD2 diplotype			
Ins-A2/ Ins-A2 compared to Ins-A1/ Ins-A1	2.2145	3.2499	0.50
Ins-A2/ Ins-A1 compared to Ins-A1/ Ins-A1	2.0831	3.5303	0.56
Ins-A2/ Del-A1 compared to Ins-A1/ Ins-A1	-3.6548	4.9034	0.46
Ins-A2/ Del-A2 compared to Ins-A1/ Ins-A1	9.4921	5.1492	0.07

Negative subscale			
Baseline PANSS total score	0.1721	0.0644	0.01
DRD2 diplotype			
Ins-A2/ Ins-A2 compared to Ins-A1/ Ins-A1	2.5207	2.6640	0.35
Ins-A2/ Ins-A1 compared to Ins-A1/ Ins-A1	-0.8251	2.8938	0.78
Ins-A2/ Del-A1 compared to Ins-A1/ Ins-A1	4.8778	4.0193	0.23
Ins-A2/ Del-A2 compared to Ins-A1/ Ins-A1	4.7164	4.2208	0.27
General psychopathology			
Switched Group compared to New Group	-3.5534	0.9942	0.001
DRD2 diplotype			
Ins-A2/ Ins-A2 compared to Ins-A1/ Ins-A1	1.7566	1.4901	0.25
Ins-A2/ Ins-A1 compared to Ins-A1/ Ins-A1	0.7854	1.6187	0.63
Ins-A2/ Del-A1 compared to Ins-A1/ Ins-A1	-0.2849	2.2483	0.89
Ins-A2/ Del-A2 compared to Ins-A1/ Ins-A1	4.5848	2.3610	0.06

Association Study of the Brain-derived Neurotrophic Factor (BDNF) Gene with Bipolar Disorder

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Summary

Brain-derived neurotrophic factor (BDNF) belongs to a family of neurotrophic factors and has been demonstrated to promote the survival, differentiation, and maintenance of a broad variety of central nervous system neurons. Several reports have suggested that the BDNF gene is a plausible functional candidate gene underlying the predisposition for developing bipolar disorder (BPD). In the present study, we investigated the possible role of the BDNF gene in the etiology of BPD using a matched case-control association design in a Japanese population. There was no evidence for an allelic or genotypic association of two polymorphisms (–1360C>T and 196G>A) of the BDNF gene with BPD. Furthermore, no significant association was observed between these polymorphisms and either of two diagnostic subtypes (bipolar I and bipolar II disorder). The results suggest that the BDNF gene is unlikely to confer susceptibility to BPD.

Bipolar disorder (BPD) is a common mental illness characterized by episodes of mania and depression. It has a lifetime prevalence of approximately 1% of the world's population. The pathophysiology and etiology of BPD remain unknown. Family, twin, and adoption studies have provided strong evidence for an important genetic component [2,13,22].

Brain-derived neurotrophic factor (BDNF) belongs to a family of neurotrophic factors that also include nerve growth factor, neurotrophin-3, and neurotrophin-4 [20]. It is most widely and abundantly expressed within the brain and has been demonstrated to promote the survival, differentiation, and maintenance of a broad variety of central nervous system neurons [1,8,9]. Several lines of evidence point to the BDNF gene as a

reasonable candidate gene for psychiatric disorders, including BPD. Repeated administration of antidepressant drugs increases the expression of BDNF in rat brain limbic regions, particularly the hippocampus [6,15,16]. Indeed, in the human brain, Chen et al. [3] have reported that BDNF levels were increased in the hippocampus of subjects treated with antidepressants. Direct infusion of BDNF into the midbrain or the hippocampus of rats is reported to produce antidepressant effects in behavioral models of depression, including the forced swim and learned helplessness paradigms [17,18]. The results of these studies indicate that BDNF may contribute to the pathophysiology of depressive disorder, and possibly the depressive symptoms associated with BPD and schizophrenia. Furthermore, electroconvulsive treatments, which are used for the treatment of depressive disorder, BPD, and schizophrenia, also increase the expression of BDNF in the frontal cortex [5,15]. Chronic administration of lithium or valproate, which are used for the treatment of BPD, increases the expression of BDNF in the cerebral cortex of the rat brain [7]. In addition, two linkage studies have suggested that chromosome 11p13-14 is a putative locus for the genes responsible for the development of BPD [4,12], and the BDNF gene is located in this region. On the basis of these evidences, we evaluated the role of the BDNF gene in BPD. In the present study, we investigated the genetic association between two different polymorphisms of the human BDNF gene and BPD through case-control studies.

One hundred and thirty-two unrelated patients with BPD (69 males and 63 females; mean age, 52.0 ± 13.9 years), including 102 with bipolar I disorder and 30 with bipolar II disorder, participated in this study. Diagnoses of BPD were made by two experienced psychiatrists, according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria and on the basis of interviews and all available information, including hospital records. One hundred and ninety healthy volunteers (98 males and 92 females; mean age, 49.1 ± 15.1 years) were recruited as control subjects, mostly from the medical staff. Within the control group, subjects with a positive personal or familial history of major psychiatric disorders were excluded. Patients and controls were unrelated Japanese, and were individually matched for gender, age, and geographical origin. After being provided with a complete description of the study, written informed consent to participate was obtained from all participants prior to examination. This study protocol was approved by the Ethics Committee of Okayama University Graduate School of

Medicine and Dentistry.

The human BDNF gene is encoded by a gene of approximately 43 kb that is located on chromosome 11p13 [11] and consists of 5 exons (MIM *113505). The first 4 exons contain putative promoter elements that control the expression of BDNF, and exon 5 contains the entire coding region for BDNF protein. In the study presented here, we investigated two kinds of single nucleotide polymorphisms (SNPs) of the human BDNF gene, –1360C>T and 196G>A. The –1360C>T polymorphism, which was detected and named C270T by Kunugi et al. [10], was localized within the 5' untranslated region (5'UTR) of exon 1. The 196G>A polymorphism was a nonsynonymous mutation that accompanied a Val66Met substitution located in exon 5. The A of the ATG-translation initiation codon is denoted nucleotide +1. The nucleotide 5' to +1 is numbered –1. Exonic SNPs are numbered according to their positions in the coding sequence.

The genomic DNA was extracted from peripheral leukocytes by standard procedures. Polymerase chain reaction (PCR) and the PCR-based restriction fragment length polymorphism (RFLP) assays were performed to genotype the DNA sequence variants of the BDNF gene. PCR was carried out in a total volume of 15 μl with 10% dimethyl-sulfoxide and 0.75 units of Super*Taq* DNA polymerase (Sawady Technology Co., Japan) in the reaction mixture. The primer sequences used for analysis of –1360C>T were the forward primer

5'-CAGAGGAGCCAGCCCGGTGCG-3'

and the reverse primer 5'-CTCCTGCACCAAGCCCCATTC-3' [10]. Those of 196G>A were 5'-ACTCTGGAGAGCGTGAATGG-3' and 5'-ACTACTGAGCATCACCCTGGA-3' [21]. The amplification conditions were initiated at 95°C for 5 min, followed by 35 cycles consisting of denaturation at 95°C for 1 min, annealing at the appropriate primer-pair annealing temperature for 30 s and extension at 72ºC for 1 min, with a final extension step of 10 min at 72°C. The PCR products were digested at 37°C with the corresponding restriction enzyme, *Hinf*I (–1360C>T) and *Eco*72I (196G>A), and subsequently electrophoresed on 3.0% agarose gels stained with ethidium bromide. Digestion with *Hinf*I generated four fragments of 127, 63, 18, and 15 bp in subjects with –1360T allele, whereas those with –1360C allele generated three fragments of 127, 78, and 18 bp. *Eco*72I digestion produced two fragments (99 and 72 bp) in subjects with the 196G allele, whereas those with the 196A allele produced only a 171 bp fragment.

The presence of the Hardy-Weinberg

equilibrium was tested using a chi-square goodness-of-fit test. The statistical significance of differences in the genotype distribution and allele frequency between patients and controls was assessed by a chi-square test or Fisher's exact test at a significance level of 0.05, two-tailed. The level of linkage disequilibrium between two SNP sites, –1360C>T and 196G>A, was analyzed using the EH program.

Both the genotype distributions and allele frequencies for the patients and controls are shown in Table 1. The genotype distributions for patient and control groups did not deviate significantly from the Hardy-Weinberg equilibrium at these polymorphic loci. No significant differences were found in the frequency of the genotype or allele in these two polymorphisms between patients and controls (–1360C>T: genotype, χ^2 = 0.79, df = 1, P = 0.38, allele, χ^2 = 0.76, df = 1, P = 0.39; 196G>A: genotype, χ^2 = 0.32, df = 2, P = 0.85, allele, χ^2 = 0.02, df = 1, P = 0.94). With regard to the subtype of BPD, no association was observed between either of these polymorphisms and any of the diagnostic subtypes, bipolar I disorder (–1360C>T: genotype, χ^2 = 0.46, df = 1, P = 0.62, allele, χ^2 = 0.44, df = 1, P = 0.63; 196G>A: genotype, χ^2 = 0.09, df = 2, P = 0.97, allele, χ^2 = 0.07, df = 1, P = 0.86) and bipolar II disorder (–1360C>T: genotype, χ^2 = 0.77, df = 1, P = 0.41, allele, χ^2 = 0.75, df = 1, P = 0.42; 196G>A: genotype, χ^2 = 1.15, df = 2, P = 0.65, allele, χ^2 = 0.03, df = 1, P = 0.89). Pair-wise linkage disequilibrium was calculated between the two SNPs using the EH program. We found that the two SNPs were not in linkage disequilibrium with each other (Control: χ^2 = 5.32, df = 3, P = 0.15; BPD: χ^2 = 5.22, df = 3, P = 0.16). Accordingly, haplotype analyses using these two SNPs were not applicapable.

This study examined the possible association of two human BDNF gene polymorphisms with BPD. We genotyped the two polymorphisms of the BDNF gene, –1360C>T and 196G>A, in a Japanese population and found no association between the BDNF gene and BPD. However, our results seem to be not consistent with two recent studies reported in 2002. Neves-Pereira et al. [14] and Sklar et al. [19] showed positive association between certain haplotype of the BDNF gene and BPD by family-based association study. Their subjects were almost Caucasian, and ours were Japanese. An ethnic difference may result in these inconsistent results. As to the 196G>A polymorphism, the 196G allele frequencies in our control samples, Neves-Pereira's samples, and Sklar's samples were 0.579, 0.769, and 0.83,

respectively. These data indicate that the allele frequency of the BDNF gene polymorphism is likely to differ between the two groups of different ethnicity. Therefore, the BDNF gene may confer a susceptibility to BPD in Caucasian, but not in Japanese population. However, for the following reasons we cannot definitely exclude the possibility of false negative results. First, there is a possibility of the low statistical power. In the present sample size, the statistical power to detect a small effect size (w = 0.11) was 0.80, considering an alpha value of 0.05, for detecting a significant difference in allelic distributions. As judged by the statistical power, the present total sample size was estimated to have been sufficient to reveal any statistically significant differences. However, with regard to the subtypes of BPD, especially bipolar II disorder, the power was dramatically reduced because of the limited sample size, and so our results must be qualified with a larger number of subjects. Secondly, the effect of population stratification must be taken into account. However, since all the subjects were unrelated Japanese, born and living in the middle western area of Japan, and were carefully matched for ethnicity and drawn from a population that was ethnically as homogeneous as possible, the failure to demonstrate an association was unlikely to be due to population stratification. Finally, it is possible that other as yet undetected variants of the BDNF gene may be involved in the pathogenesis of BPD. In the present study, we investigated only two polymorphisms within the 5'UTR and coding region of the BDNF gene. Therefore, it remains possible that other sequence variations in, for instance, the promoter or yet undetected 3'UTR regions, may be of importance in determining susceptibility to BPD. In addition, we found that -1360C>T and 196G>A were not in linkage disequilibrium with each other. We believe that this result is reliable because these polymorphisms were approximately 42 kb apart. However, we cannot exclude the possibility that there are other unknown polymorphisms located between -1360C>T and 196G>A, which are not in linkage disequilibrium with these two polymorphisms and confer susceptibility to BPD.

In conclusion, the results of this study do not support a possible association of the BDNF gene with susceptibility to BPD. Further studies are required to clarify whether any as yet unidentified functional mutation in the BDNF gene is involved in the etiology of BPD.

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Gene Expression Related to Synaptogenesis, Neuritogenesis, and MAP Kinase in Behavioral Sensitization to Psychostimulants

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Abstract

The most important characteristic of behavioral sensitization to psychostimulants, such as amphetamine and cocaine, is the very long-lasting hypersensitivity to the drug after cessation of exposure. Re-arrangement and structural modification of neural networks in CNS must be involved in behavioral sensitization. Previous microscopic studies have shown that the length of dendrites and density of dendritic spines increased in the nucleus accumbens and frontal cortex after repeated exposure to amphetamine and cocaine, but the molecular mechanisms responsible are not well understood. We investigated a set of genes related to synaptogenesis, neuritogenesis, and mitogen-activated protein (MAP) kinase after exposure to methamphetamine. *Synaptophysin* mRNA, but not *VAMP2* (*synaptobrevin 2*) mRNA, which are considered as synaptogenesis markers, increased in the accumbens, striatum, hippocampus, and several cortices, including medial frontal cortex, after a single dose of 4 mg/kg methamphetamine. *Stathmin* mRNA, but not *neuritin* or *narp* mRNA, which are markers for neuritic sprouting, increased in the striatum, hippocampus and cortices after a single dose of methamphetamine. The mRNA of *arc*, an activity regulated protein associated with cytoskeleton, but not of *alpha-tubulin*, as markers for neuritic elongation, showed robust increases in the striatum, hippocampus, and cortices after a single dose of methamphetamine. The mRNAs of *MAP kinase phosphatase-1 (MKP-1)*, *MKP-3*, and *rheb*, a ras homologue abundant in brain, were investigated to assess the MAP kinase cascades. *MKP-1* and *MKP-3* mRNAs, but not rheb mRNA, increased in the striatum, thalamus, and cortices, and in the striatum, hippocampus, and cortices, respectively, after a single methamphetamine. *Synaptophysin* and *stathmin* mRNAs did not increase again after chronic methamphetamine administration, whereas the increases in *arc*, *MKP-1* and *MKP-3* mRNAs persisted in the brain regions after chronic methamphetamine administration. These findings indicate that the earlier induction process in behavioral sensitization may require various plastic modifications, such as synaptogenesis, neuritic sprouting, neuritic elongation, and activation of MAP kinase cascades, throughout the almost the entire brain. In contrast, later maintenance process of sensitization may require only limited plastic modification in restricted regions.

1. Introduction

Behavioral sensitization or reverse tolerance induced in experimental animals by administration of psychostimulants, including methamphetamine, amphetamine, and cocaine has been recognized and used in animal models of human methamphetamine psychoses and schizophrenia[1,2]. The rationale for so doing is based on the striking analogy between them, especially in the chronological quantitative and qualitative alterations in the response to psychostimulants. For example, repeated administration of a fixed dose of psychostimulants to rodents induces progressive augmentation in induced abnormal behaviors, such as increased hyperlocomotion and characteristic stereotypes. Such enhanced responses to drugs must be due to the development of altered function, supersensitivity, in the host brain. Similar quantitative augmentation and qualitative alterations in response to psychostimulants is commonly observed in human methamphetamine psychoses[3,4]. Abuse of methamphetamine induces euphoria and hyperarousal in the user, but further repeated use induces gradual development of psychotic states, mainly characterized by acoustic hallucinations and delusions. The psychotic symptoms induced by methamphetamine recover relatively rapidly by discontinuation of use, but they relapse readily after resumption of the drug, or sometimes in response to alcohol consumption or psychological stress (cross-sensitization). The susceptibilityto relapse into psychostimulant-induced psychoses persists even after long-term abstinence, are suspected of being the same as in the mechanisms underlying susceptibility to relapse seen in chronic schizophrenics. The most important characteristic of the sensitization phenomenon is the very long persistence of hyperresponsiveness to the drugs. Rats previously sensitized to methamphetamine remain hypersensitive to the psychomotor activating and rewarding effects of a subsequent dose after at least 6 months of abstinence[5]. When the life span of rats is considered, sensitization to the drug seems to be almost perpetual.

Various neurochemical adaptations have been found in the sensitization phenomenon, including up- or down-regulation of D1 dopamine[6], sigma[7-9], and neuropeptide receptors[10-12], altered dopamine transporters mRNA[13], changes in the alpha and beta subunits of trimeric G proteins[14-16] and enzymes for dopamine synthesis and metabolism[2], increased adenylyl cyclase activity, cyclic AMP and protein kinase A[17], increased calmodulin and activated CamK II[18-20], and increased c-fos and AP-1 binding protein[21,22]. However, since these subcellular neurochemical adaptations are all transient or reversible, they must be converted to more lasting plastic brain changes during sensitization. Previous studies have shown that the protein synthesis inhibitors, anisomycin and cycloheximide, block the development of

sensitization[23,24], and that pups younger than 3 weeks old, who are too immature to develop neural networks, do not become sensitized to psychostimulants[25,26]. This evidence suggests that re-arrangement of neural networks and circuitry must develop for the long-lasting plasticity required for sensitization to occur.

Lines of evidence from previous behavioral pharmacological studies have suggested that re-arrangement of the neural networks shown in Fig 1[27] must develop during the establishment of behavioral sensitization. The A10 dopaminergic pathway from the ventral tegmentum area (VTA) to mesolimbic areas, such as the nucleus accumbens and medial prefrontal cortex, plays an indispensable role in the development of psychostimulant-induced sensitization, it is also called the "rewarding system". Psychostimulants enhance dopamine release from the synaptic terminals of A10 dopamine neurons in the accumbens, and the "pleasant" or "high feeling" signals are transmitted to the PPT via the ventral pallidum, and ultimately result in drug-seeking behavior and abnormal stereotypy. The output signals from the accumbens may be highly regulated by the glutaminergic afferents from prefrontal cortex, amygdala, and hippocampus. Increased glutamate release in the accumbens also enhanced abnormal stereotyped behaviors. Thus, the VTA - accumbens - frontal cortex - accumbens - VTA circuitry may be constructed and strengthened during the development of sensitization and persisted for very long period. Moreover, microscopic studies with Golgi-staining have demonstrated anatomical changes as a result of exposure to psychostimulants corresponding to such enhanced synaptic connectivity or transduction efficacy in the circuitry[28,29]. Chronic exposure to AMP or cocaine produces an increase in the length of dendrites, the density of dendritic spines, and the number of branched spines on the major output cells of the accumbens and prefrontal cortex, but the precise molecular basis for such morphological changes in response to exposure to psychostimulants is not well understood. To further clarify it, in this study we used *in situ* hybridization and western blots to assess a set of genes for synaptogenesis, neuritogenesis, and mitogen-activating protein (MAP) kinases, which are involved in very diverse plasticity.

2. Genes related to synaptogenesis for the sensitization phenomenon

The genes *synaptophysin*[30] and *VAMP-2* (also called as *synaptobrevin 2*)[31] were examined as markers for presynaptic plasticity and synaptogenesis. The molecules encoded by these genes are major integral membrane proteins of small presynaptic vesicles that participate in neurotransmitter exocytosis, and they are molecular indicators for synaptic densities. In naive rat brain, synaptophysin mRNA is most densely distributed in the hippocampus, whereas its density is moderate in parts of the cortex, such as the temporal cortex, parietal cortex, and occipital cortex, and minimal in the prefrontal

cortex, striatum, accumbens, and amygdala (fig. 2). One 4 mg/kg dose of methamphetamine significantly increased synaptophysin mRNA in the prefrontal cortex by about 50% 1 h later, in the temporal cortex 30 min later, in the striatum 1 h later, and in the occipital cortex, CA1 and CA3 region of hippocampus, and accumbens 3 h later [32] (fig. 3). The increase in mRNA synaptophysin in all regions subsided within 6-24 h after methamphetamine administration. By contrast, VAMP-2 mRNA was unchanged after an acute dose of methamphetamine. After chronic exposure to methamphetamine for 10 days, synaptophysin mRNA was no longer increased in any of the brain regions where increases h.d been observed after acute methamphetamine administration. These findings indicated that thr role of synaptophysin in methamphetamine-induced sensitization differs in its early and late phase, in other words, in the induction phase and expression/establishment phase of sensitization. The finding that synaptophysin mRNA increased during the process of induction of sensitization not only in the accumbens and striatum, but also in unexpectedly broad areas of several regions of the cortex, including the prefrontal cortex and the hippocampus, was significant, because it implied that widespread synaptogenesis throughout the brain occurs in the early phase of the sensitization process. Previous studies have also revealed alteration of synapse-related molecules after psychostimulant exposure, including an increase in synaptotagmin IV mRNA[33], a synaptic vesicle protein participating in Ca^{2+}-dependent and Ca^{2+}-independent interaction during membrane trafficking, in the striatum after cocaine administration. These findings indicate that increases in presynapse markers are not specific to the action of methamphetamine, but a common event after psychostimulant exposure. Increased synapse synthesis may reinforce synaptic connections and enhance neural transduction. Since the extent of synaptic vesicle protein correlates with the extent of neurotransmitter exocytosis, it may also contribute to molecular mechanisms underlying the enhanced dopamine release in the accumbens, striatum, and frontal cortex occurring after sensitization phenomenon. Another study showed that phosphorylation of synapsin I, another synaptic vesicles docking protein, increased after repeated amphetamine adminsistration[20,34]. Phosphorylated synapsin I, an activated form, renders dopamine pool in vesicles prone to release. Although methamphetamine-induced dopamine release is mainly caused by its action on the dopamine transporter, it has been suggested that Ca^{2+}-dependent and impulse-dependent exocytosis is also involved[35]. Thus, presynaptic modification by the above three molecules must develop after psychostimulant administration, and it may contribute, at least partially, to enhanced dopamine release from the presynaptic terminals of the mesocorticolimbic and nigrostriatal projection after the sensitization phenomenon.

3. Genes related to neurite sprouting in the sensitization phenomenon

Presynaptic modifications are insufficient for re-arrangement of the neural networks, and corresponding postsynaptic modifications are needed. Three molecules, stathmin, narp, and neuritin, were examined as markers of neurite sprouting, the first step in neuritogenesis. Stathmin is a growth-associated cytosolic phosphoprotein present in the growth cones of neurite ends and is involved in neural plasticity[36,37]. Narp belongs to the pentraxin protein family and is expressed in neurites after neural activity[38]. Narp is thought to be involved in clustering receptors, such as AMPA glutaminergic receptors (receptor clustering enhances exponentially synaptic conduction)[39]. Neuritin is a membrane protein and induces neurite branching[40].

The distribution of stathmin mRNA in naive rat brain was found to be highest in dentate gyrus of the hippocampus and almost entire cortex, aof moderate density in the CA1 and CA3 areas of the hippocampus, and of minimal density in the striatum, accumbens, and amygdala (fig 2.). Acute methamphetamine administration increased mRNA stathmin by about 30% in the prefrontal cortex, striatum, accumbens, temporal cortex, and hippocampus CA1 0.5-1 h after the injection, and it returned to the basal level within 3 h [32] (fig. 3). Neither narp nor neuritin mRNA changed after a single methamphetamine dose.

Stathmin is a cytoplasmic phosphoprotein that acts as a growth-associated protein (GAP) , and it is involved in plastic adaptation, including regulation of cell proliferation and differentiation[36,41]. The stathmin family is composed of stathmin, SCG 10[41,42], and two recently discovered molecules, RB3[43] and SCLIP[44], all of which have been shown to be involved in plasticity. Stathmin binds to microtubules and inhibits their assembly, resulting in neuritogenesis through the regulation of dynamic microtubule instability[45]. The increase in stathmin mRNA in widespread brain areas after acute methamphetamine almost coincided with the increase in synaptophysin mRNA described above. These findings may indicate that robust neurite sprouting at postsynaptic sites during the early phase of sensitization occurs in concert with synaptogenesis at presynaptic sites, resulting in robust and new generation of synaptic connections.

After chronic methamphetamine exposure, stathmin mRNA showed a slight decrease in the striatum and accumbens, but not in other brain regions. Another study, however, showed that phosphorylated form of the GAP molecule, neuromodulin (GAP-43), which is its active form, was increased in the striatum after chronic amphetamine administration[34,46]. Thus, neuromodulin, not stathmin, seems to be involved in neurite plasticity in the late phase of stimulant-induced sensitization.

4. Genes related to neuritic elongation in the sensitization phenomenon

The development of new neural networks requires the outgrowth and elongation of axons and dendrites, and this must be accompanied by increased synthesis of neuritic components. Since alpha-tubulin is a cytoskeletal protein and component of microtubles[47], and arc is an activity-regulated cytoskeleton-associated protein that is localized in the perikarya and dendritic processes and co-sedimentates with actin[48], their mRNAs were examined as markers for neuritic elongation.

After a single dose of methamphetamine adminsitration, arc increased sharply by 70-150% in the frontal cortex, orbital cortex, and cingulate cortex, and by 20-50% in the striatum and hippocampus Kodama, 1997 #11] (fig. 2). These increases reached peaked around 1 h after the methamphetamine injection, and the values returned to their basal levels by 6 h. The increases in arc mRNA in every brain region after methamphetamine injection were reversed by co-administration of SCH 23390, a D1 dopamine antagonist, or MK-801, a NMDA antagonist, and both compounds, SCH 23390 and MK-801, have been shown to prevent the development of sensitization to psychostimulants[49,50]. Microscopic autoradiography revealed that increased arc mRNAs after methamphetamine exposure in the parietal cortex was abundant in layers IV and VI, and those in the striatum existed mainly in the medium-sized neurons[51]. The D1 receptors in the cortex are mostly distributed in layer VI, which receives mesocortical dopaminergic projections. Accordingly, the enhanced dopamine release induced by methamphetamine may activate arc transcription via D1 dopamine receptors, at least in cortical layer VI. After chronic methamphetamine exposure, the arc mRNA response to a methamphetamine challenge was similar to that seen after acute methamphetamine administration and occurred in similar brain regions. These results suggested that every methamphetamine dose, regardless of whether acute or chronic, stimulates arc synthesis widely in the brain, including in the hippocampus, corticostriatal, and striatocortical projections, and this may indicate neurite outgrowth and elongation in those areas.

Contrary to our expectations based on the arc findings, the alpha-tubulin mRNA level was constant after methamphetamine exposure[32], which may mean that the amount of microtubulin was constant. Since disengagement and polymerization of alpha-tubulin plays a role in axonal and dendritic outgrowth, polymerization of alpha-tubulin, rather than increased synthesis, may be important for neuritogenesis in the sensitization phenomenon. Alternatively, other components of microtubules, for example, microtubule-associated proteins, may be more important for the neural plasticity during sensitization.

5. Genes related to regulation of MAP kinase in the sensitization phenomenon

The MAP kinase cascades have been shown to play a crucial role in various types of