until used. The brain sections were permeabilized with 0.1% Triton X-100, blocked with 1% bovine serum albumin and incubated with the mouse monoclonal antibody against GDNF (R&D Systems, USA) at 4 °C overnight. To detect specific signals, the brain sections were incubated with CF594-conjugated secondary antibodies (Biotium, USA) for 2 h at room temperature. Images were captured and the density was evaluated with fluorescence microscopy (BZ9000; Keyence, Japan).

### *Meth self-administration, extinction and reinstatement of Meth-seeking behaviour*

*Apparatus*

The standard mouse operant conditioning chambers (ENV-307A; Med Associates, USA) used in the current study were described previously (Yan et al., 2006, 2007a; Yan and Nabeshima, 2009).

*Meth self-administration*

After a 2-wk interval from the microinjection of the AAV-Gdnf or AAV-EGFP vectors into the striatum, the mice were subjected to daily 3-h sessions of Meth self-administration under a fixed ratio (FR) schedule of reinforcement. Throughout each session of self-administration, the house lights were illuminated and cue- and hole-lamps indicated the availability of Meth. Once the mice made nose-poke responses in the active hole, the cue- and hole-lamps were turned off and Meth (0.1 mg/kg.infusion) was delivered over 5 s followed by a 5-s time-out period. Responses in the active hole during the time-out period and in the inactive hole had no programmed consequences but were recorded. Self-administration was initially under an FR1 schedule of Meth reinforcement. Once the mice made 60% active nose pokes on average, an FR2 schedule of Meth reinforcement was introduced until the mice acquired stable Meth self-administration behaviour (deviations of $<15\%$ of the mean of active responses in three consecutive training sessions).

*Extinction*

The mice were then subjected to 8–16 daily 3-h sessions of extinction before the Meth-primed reinstatement test and then 3–6 daily 3-h sessions of extinction before the cue-induced reinstatement test until they met the extinction criterion ($<15$ active responses or 25% of active responses in the stable phase of self-administration in two consecutive sessions). The number of extinction training sessions for each mouse largely varied in the same treatment of group. Throughout the extinction session, the house light was on. The Meth-associated cue- and hole-lamps, and the pump for Meth infusion, were turned off. Therefore, nose-poke responses into the previously active hole resulted in neither an infusion of Meth nor Meth-associated cues (cue- and hole-lamps and pump noise for Meth infusion).

*Meth-primed reinstatement*

Once the extinction criterion was met, the animals were first subjected to a 3-h session of the operant test 30 min after the injection (i.p.) of saline as a control for the Meth-primed reinstatement. From the next day, the mice were subjected to daily 3-h tests for Meth-primed reinstatement 30 min after the i.p. injection with increasing doses of Meth (0.2, 0.4, 1.0 or 2.0 mg/kg, each dose for one daily 3-h session). All of mice were tested with each dose of Meth for drug-primed reinstatement on different days, but there was no extinction training between the tests. This is because: (1) different to drug-primed instatement in rats, drug-primed reinstatement in mice is transient; (2) it takes a much longer time for mice to be extinguished from drug self-administration than that in rats. The Meth-primed reinstatement tests were conducted under the same conditions as in the extinction sessions, in which neither Meth infusions nor Meth-associated cues were available after nose-poke responses into a previously active hole. Nose-poke responses in the previously active or inactive hole were counted as active and inactive, respectively.

*Cue-induced reinstatement*

After testing Meth-primed reinstatement, the same group of animals was subjected to extinction training once again. Once the extinction criterion was met, the animals were subjected to cue-induced reinstatement for the first time (the first test). Two months later, the same group of animals was subjected to cue-induced reinstatement for the second time (the second test). The cue-induced reinstatement tests were conducted under the same conditions as the Meth self-administration under the FR2 schedule, except that Meth was unavailable throughout the testing session. Nose-poke responses in the previously active or inactive hole were counted as active and inactive, respectively.

### *Data analysis*

All data were expressed as the mean $\pm$ S.E.M. The data of GDNF densities, locomotor activities and total Meth intake between AAV-Gdnf and AAV-EGFP-treated mice were analysed with Student's $t$ test. A two-way analysis of variance (ANOVA) with (or without) repeated measures was performed for the difference in either active or inactive nose-poke responses between the AAV-Gdnf and AAV-EGFP-treated mice during Meth self-administration, extinction training, Meth-primed and cue-induced reinstatement of Meth-seeking behaviour, followed *post hoc* by Bonferroni's multiple

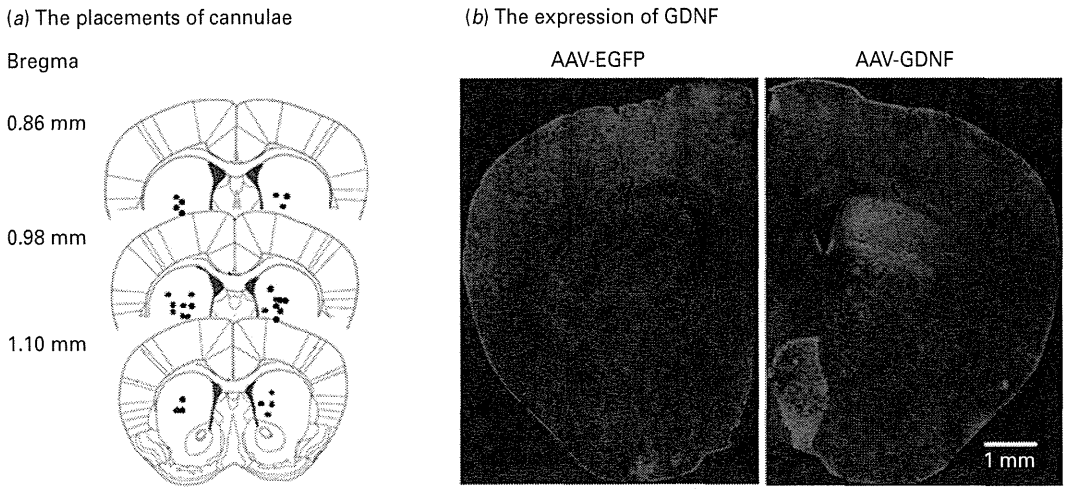


**Fig. 1.** Expression of GDNF in adeno-associated virus-mediated glial cell line-derived neurotrophic factor (AAV-GDNF)-treated mice. (*a*) Illustrates the placements of injectors within the mouse brain; (*b*) indicates GDNF protein expression in the dorsal striatum of AAV-GDNF- and adeno-associated virus-mediated enhanced green fluorescent protein (AAV-EGFP)-treated mice.

comparison test. In all cases, a significant difference was set at $p<0.05$.

## Results

### *Enhancement of GDNF expression in the dorsal striatum of the AAV-Gdnf-treated mice*

Figure 1*a* indicates the placement of cannulae for the intrastriatal microinjection of the AAV-Gdnf or AAV-EGFP vectors into the mouse brain. Figure 1*b* shows that the expression level of GDNF protein was clearly enhanced in the striatum 2 wk after the intrastriatal microinjection of the AAV-Gdnf vectors as compared to that after the microinjection of AAV-EGFP vectors. The densities of GDNF expression were $47.1\pm0.35$, $137.1\pm5.06$ in the striatum of AAV-EGFP and AAV-Gdnf, respectively, indicating that GDNF content increased significantly in the striatum by microinjection of AAV-Gdnf (Student's $t$ test, $p<0.001$, d.f.$=41$, $t=4.08$). To investigate the effects of the intrastriatal microinjection of AAV-Gdnf and AAV-EGFP vectors on behavioural performance in general, the motility in a novel environment was measured for locomotion and rearing, as the motor issue and exploratory motivation, respectively. Neither locomotion nor rearing during a 60-min period of observation differed significantly between the AAV-Gdnf (locomotion: $28223.7\pm1978.0$ counts; rearing: $526.5\pm52.9$ counts, $N=6$) and AAV-EGFP-treated mice (locomotion: $24539.6\pm976.5$ counts; rearing: $542.4\pm49.9$ and $526.5\pm130.0$ counts, $N=6$; Student's $t$ test, $p=0.94$, d.f.$=10$, $t=-1.67$ for locomotion; $p=0.415$, d.f.$=10$, $t=0.220$ for rearing). These results indicate that microinjection of AAV-Gdnf has no significant influence on the general locomotion and exploratory motivation system in mice.

### *Attenuation of Meth self-administration behaviour in AAV-Gdnf-treated mice*

Active and inactive nose-poke responses of AAV-Gdnf and AAV-EGFP-treated mice during Meth self-administration training are shown in Fig. 2*a*. Repeated two-way ANOVA analysis for active nose-poke responses (AAV vectors are between-subjects factors and training sessions are within-subjects factors) revealed that there was no significant difference in active nose-poke responses in the early phase of Meth self-administration (day 1–11) between the AAV-Gdnf and AAV-EGFP-treated mice. In the late phase of Meth self-administration, however, the active nose-poke responses to take Meth were lower in the AAV-Gdnf-treated mice than in the AAV-EGFP-treated mice (main effect of AAV vectors: $F_{1,14}=3.94$, $p<0.05$; main effect of training sessions: $F_{15,210}=28.39$, $p<0.001$; AAV vector × training session interaction: $F_{15,210}=2.31$, $p<0.01$). There was no significant difference in inactive nose-poke responses between the AAV-Gdnf and AAV-EGFP-treated mice throughout Meth self-administration training (day 1–16). We have previously reported that total intake of Meth during drug self-administration affects the subsequent Meth-primed reinstatement (Yan et al., 2007a). After both groups of animals acquired stable Meth self-administration, the AAV-Gdnf-treated mice continued to be subject to Meth self-administration for four additional sessions to make two groups of animals with an equivalent total intake of Meth during drug self-administration training (Fig. 2*a*, day 17–20). As shown in Fig. 2*b*, the total intake of Meth during drug self-administration was $23.03\pm3.09$ mg/kg in AAV-Gdnf-treated mice for 20 d and $22.86\pm3.22$ mg/kg in AAV-EGFP-treated mice for 16 d (Student's $t$ test, $p=0.97$, d.f.$=14$, $t=0.04$). These observations suggest that the intrastriatal microinjection

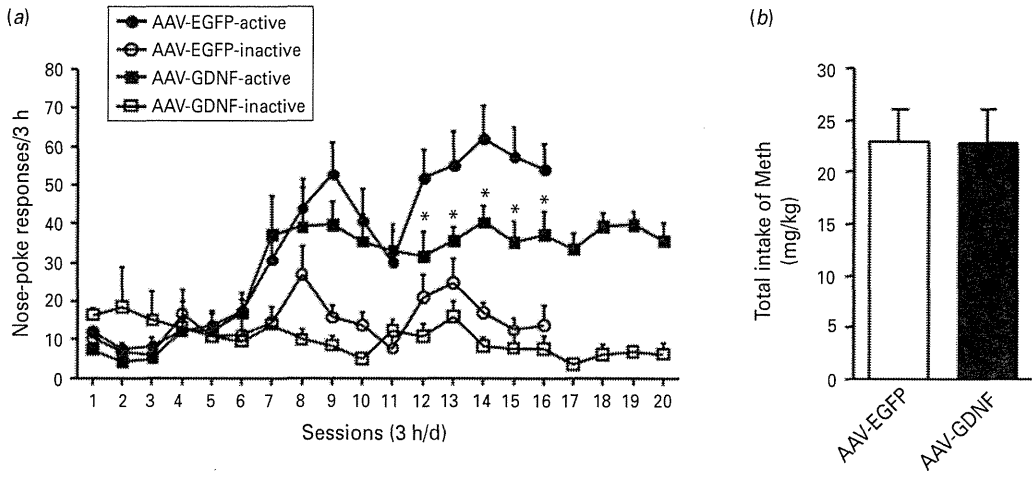


**Fig. 2.** Acquisition of methamphetamine (Meth) self-administration behaviour and total intake of Meth in adeno-associated virus-mediated glial cell line-derived neurotrophic factor (AAV-GDNF) and adeno-associated virus-mediated enhanced green fluorescent protein (AAV-EGFP)-treated mice. (*a*) Indicates the number of active and inactive nose-poke responses during Meth self-administration. * $p<0.05$ *vs.* active nose-poke responses of AAV-EGFP-treated mice on the same training day. (*b*) Indicates the total intake of Meth during Meth self-administration training in AAV-GDNF and AAV-EGFP-treated mice (23.03±3.09 mg/kg for 20 d and 22.86±3.22 mg/kg for 16 d, respectively). Data are presented as the mean±S.E.M. and $N=7$–8 for each group.

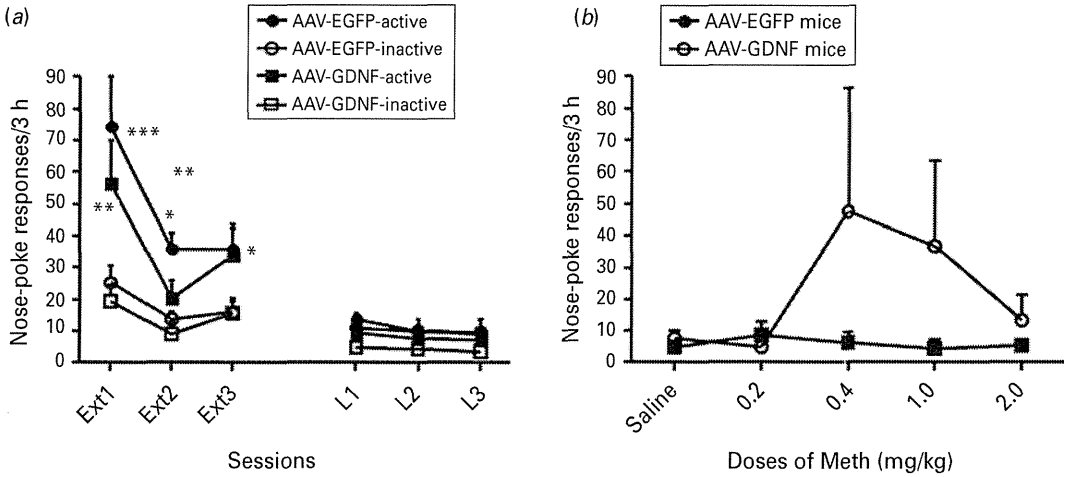


**Fig. 3.** Extinction performance and methamphetamine (Meth)-primed reinstatement of drug-seeking behaviour in adeno-associated virus-mediated glial cell line-derived neurotrophic factor (AAV-GDNF) and adeno-associated virus-mediated enhanced green fluorescent protein (AAV-EGFP)-treated mice. (*a*) Indicates nose-poke responses during the extinction training. The data are from the first three daily 3-h sessions (indicated by Ext1–3) and the last three daily 3-h sessions (indicated by L1–L3) during 8–16 extinction training sessions before the test for Meth-primed reinstatement. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ *vs.* the corresponding inactive nose-poke responses in the same group. (*b*) Indicates the active nose-poke responses during Meth-primed reinstatement between AAV-Gdnf and AAV-EGFP-treated mice. Data are presented as the mean±S.E.M. and $N=7$–8 for each group.

of the AAV-Gdnf vectors is effective to attenuate the late phase of Meth self-administration behaviour in mice.

### *No difference in the process of extinction, but decrease of Meth-primed reinstatement in the AAV-Gdnf-treated mice*

After the above-mentioned Meth self-administration, the same two groups of mice were subjected to 8–16 daily 3-h sessions of extinction training. As shown in Fig. 3*a*, repeated two-way ANOVA for active *vs.* inactive nose-poke holes in the same AAV vector treatment revealed that both groups of mice exhibit higher active than inactive nose-poke responses at the early phase of extinction training (for AAV-Gdnf-treated mice, main effect of within-subjects factor nose-poke holes: $F_{1,14}=9.64$, $p<0.01$; main effect of within-subjects factor training sessions: $F_{5,70}=14.03$, $p<0.001$; nose-poke hole × training session interaction: $F_{5,70}=3.44$, $p<0.01$. For AAV-EGFP-treated mice, main effect of within-subjects factor

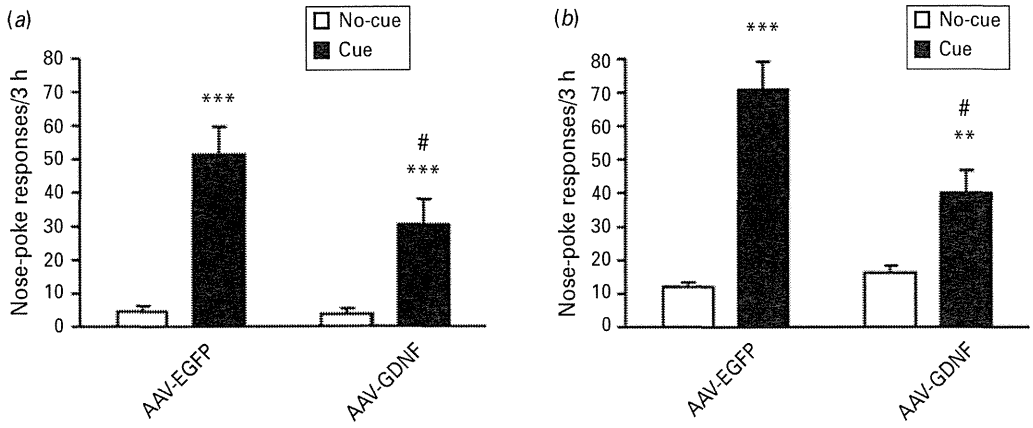


**Fig. 4.** Cue-induced reinstatement of methamphetamine (Meth)-seeking behaviour in adeno-associated virus-mediated glial cell line-derived neurotrophic factor (AAV-GDNF) and adeno-associated virus-mediated enhanced green fluorescent protein (AAV-EGFP)-treated mice. (*a*) Indicates cue-induced relapsing behaviour for the first test (the extinction criteria met after the test for Meth-primed reinstatement). *** $p<0.001$ *vs.* the no-cue condition, # $p<0.05$ *vs.* AAV-EGFP-treated mice. (*b*) Indicates the second test for cue-induced relapsing behaviour (2 months after the first test for cue-induced reinstatement). *** $p<0.001$ *vs.* the no-cue condition, # $p<0.05$ *vs.* AAV-EGFP-treated mice. Data are presented as the mean ± S.E.M. and $N=7$–8 for each group. No-cue, Control for the reinstatement test (neither Meth-associated cues nor Meth infusion); Cue, Meth-associated cue-induced reinstatement (with Meth-associated cues but no Meth infusion).

nose-poke holes: $F_{1,14}=8.45$, $p<0.01$; main effect of within-subjects factor training sessions: $F_{5,70}=17.95$, $p<0.001$; nose-poke hole × training session interaction: $F_{5,70}=6.99$, $p<0.001$). However, there was no significant difference in active nose-poke responses between the AAV-Gdnf and AAV-EGFP-treated mice throughout extinction training (main effect of between-subjects factor AAV vectors: $F_{1,14}=1.82$, $p=0.20$; main effect of within-subjects factor training sessions: $F_{5,70}=21.99$, $p<0.001$; AAV vector × training session interaction: $F_{5,70}=0.61$, $p=0.69$). There was no significant difference in the number of sessions (d) taken for extinction training between the AAV-Gdnf and AAV-EGFP-treated mice (data not shown). When the extinction criteria met, the two groups of mice were subjected to testing for Meth-primed reinstatement of drug-seeking behaviour. As shown in Fig. 3*b*, the AAV-EGFP-treated mice showed a clear dose-dependent tendency for drug-seeking behaviour induced by the i.p. priming injection of Meth. In contrast, the AAV-Gdnf-treated mice failed to show Meth-seeking behaviour after the priming injection of Meth at all doses examined (0.2–2.0 mg/kg i.p.). These data indicate that the intrastriatal microinjection of the AAV-Gdnf vectors may also be effective to attenuate Meth-primed reinstatement of Meth-seeking behaviour in mice.

### *Long-lasting inhibition of cue-induced relapsing behaviour in the AAV-Gdnf-treated mice*

After testing for Meth-primed reinstatement, the same two groups of mice were subjected to 3–6 daily 3-h sessions of extinction training until the extinction criteria were met. When exposed to previous Meth-associated cues, both groups of mice showed cue-induced relapsing behaviour (Fig. 4*a*, two-way ANOVA, main effect of within-subjects factor cue and no-cue factors: $F_{1,26}=38.82$, $p<0.001$). Importantly, the number of active nose-poke responses was significantly reduced in the AAV-Gdnf-treated mice as compared to those in the AAV-EGFP-treated mice (Fig. 4*a*, two-way ANOVA, main effect of between-subjects factor AAV-Gdnf and AAV-EGFP treatments: $F_{1,26}=3.44$, $p<0.05$; cue × treatments interaction: $F_{1,26}=2.97$, $p=0.10$). Two months after this testing, the same two groups of mice were subjected to extinction training once again until the criteria were met. As shown in Fig. 4*b*, cue-induced reinstatement was significantly attenuated in the AAV-Gdnf-treated mice as compared to that in the AAV-EGFP-treated mice (Fig. 4*b*, two-way ANOVA, main effect of between-subjects factor AAV-Gdnf and AAV-EGFP treatments: $F_{1,26}=5.55$, $p<0.05$; cue × treatments interaction: $F_{1,26}=10.11$, $p<0.01$), although both groups of mice still showed cue-induced reinstatement of Meth-seeking behaviour (Fig. 4*b*, two-way ANOVA, main effect of within-subjects factor, cue and no-cue factors: $F_{1,26}=56.01$, $p<0.001$). These findings suggest that the inhibitory effects of intrastriatal AAV-Gdnf vectors on cue-induced reinstatement of Meth-seeking behaviour are long-lasting.

## Discussion

GDNF has been considered as a potential therapeutic molecule to treat drug addiction (Ron and Janak, 2005; Niwa et al., 2008) and the AAV vectors are one of the most attractive gene delivery vehicles into the brain for the treatment of neurological diseases (Miyazaki et al., 2012). In our study, AAV-mediated delivery of a *Gdnf* gene into the striatum increased GDNF protein

expression without activation of spontaneous locomotion (Fig. 1*b*). The increased GDNF significantly attenuated Meth self-administration. Moreover, the AAV-Gdnf vectors in the striatum persistently reduced cue-induced relapsing behaviour. In addition, this manipulation also showed a clear tendency to block Meth-primed reinstatement in mice. These findings suggest that the manipulation of GDNF expression via the AAV vectors may be valuable in a clinical setting for the treatment of drug addiction and relapse.

The AAV vectors have unique characteristics, including the lack of any disease associated with the wild-type virus, an ability to infect non-dividing cells, long-term transgene expression with a minimal inflammatory or immune response and the physical stability of viral particles (Miyazaki et al., 2012). Using the AAV vectors, several research groups have constructed different versions of the AAV vectors for the Gdnf gene transfer, most of which express functional GDNF protein in a sustained manner after local injections and produce a functional recovery of the impaired dopaminergic system in the brain (Mandel et al., 1997; Wang et al., 2002; Eberling et al., 2009; Kells et al., 2010). Consistently, our previous studies have demonstrated that the expression of GDNF protein driven by an AAV-mediated Gdnf vector could be detected in the striatum from week 2 after local injection to lifetime. Furthermore, the GDNF protein could be retrogradely transported to the dopaminergic neuron cell bodies in substantial nigra from the terminals in the striatum 4 wk after the injection. The nigral dopaminergic neurons are prevented from progressive degeneration, thereby contributing to behavioural improvement in a rat model of Parkinson's disease (Wang et al., 2002). In the current study, the inhibitory effects of the AAV-Gdnf vectors into the striatum on cue-induced relapsing behaviour were sustained for at least 2 months in mice. Such persistently inhibitory effects of the AAV-mediated delivery of a *Gdnf* gene on cue-induced relapsing behaviour may result from a sustained expression of the AAV-Gdnf vectors in the nigra-striatal circuit after bilateral intrastriatal microinjection. This observation is consistent with our previous findings that $Gdnf^{+/-}$ mice show an enduring vulnerability to cue-induced reinstatement of Meth-seeking behaviour (Yan et al., 2007b). In the present study, the bilateral intrastriatal injection of the AAV-Gdnf vectors also decreased the late phase of Meth self-administration in mice (Fig. 2*a*).

It is unlikely that persistent inhibitory effects of bilateral intrastriatal injection of the vector-mediated delivery of a *GDNF* gene on Meth self-administration and cue-induced reinstatement result from non-specific procedures of microinjection. First, there was no significant difference in locomotion and rearing after the bilateral intrastriatal injection between AAV-Gdnf- and AAV-EGFP-treated mice. Second, there was no difference in extinction after the bilateral intrastriatal injection between AAV-Gdnf- and AAV-EGFP-treated mice. This observation, however, seems to be in discrepancy with one previous report in which microinjection of an AAV-Gdnf vector into the ventral tegmental area potentiates extinction responding in Long Evens rats (Lu et al., 2009). One parsimonious explanation is that the difference may reflect a distinct role of the nigrostriatal pathway or ventral tegmental area–nucleus accumbens in extinction responding (injection into the striatum in our study *vs.* injection into the ventral tegmental area in the report of Lu et al. 2009). Chen et al. (2008) have recently reported that the expression of GDNF protein via an AAV-Gdnf vector in the dorsal striatum prevents neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced deficits in the striatal synaptic plasticity. These findings may provide a potential molecular mechanism by which bilateral intrastriatal injection of the AAV-Gdnf vectors attenuated cue-induced reinstatement of Meth-seeking behaviour in our study, since it is well known that neurotoxic effects of Meth in the brain play an important role in the development of Meth addiction.

It has been well established that the nucleus accumbens and striatum have a distinct role in the development of drug addiction. The nucleus accumbens is well known to mediate the reinforcing effects of addictive drugs, whereas the striatum is critical to the transition from initial drug use to habitual drug abuse to compulsion (Everitt and Robbins, 2005). It has been postulated that, during the development of drug self-administration, neutral drug-conditioned environmental cues acquire a reinforcing property, which evokes drug craving and relapse. Previous studies have shown that the striatum is critical for cue-induced reinstatement of drug-seeking behaviour in animals (Di Ciano et al., 2008). Consistently, bilateral intrastriatal injection of the AAV-Gdnf vectors persistently attenuated cue-induced reinstatement of Meth-seeking behaviour in mice. This phenomenon may reflect a specific role of the nigra-striatal dopaminergic transmission pathway in the cue-induced relapse or the late stage of drug dependence/addiction. In addition, previous studies have shown that over-expression of GDNF in the striatum and nucleus accumbens attenuates cocaine self-administration behaviour in rats (Green-Sadan et al., 2005). In agreement with these findings, in our current study, bilateral intrastriatal injection of the AAV-Gdnf vectors significantly reduced the late phase of Meth self-administration or potentially blocked Meth-primed reinstatement.

Taken together, the bilateral intrastriatal microinjection of the AAV-Gdnf vectors in the brain significantly attenuated Meth self-administration and cue-induced reinstatement of Meth-seeking behaviour in mice, without affecting either general locomotor activity or extinction. This suggests that increased expression of exogenous GDNF protein through the microinjection of AAV-Gdnf vectors in the brain may be a gene therapeutic strategy to treat drug dependence and relapse in a clinical setting.

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**Statement of Interest**

None.

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Clinical Observations

# A Three-Year-Old Boy With Glucose Transporter Type 1 Deficiency Syndrome Presenting With Episodic Ataxia

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ABSTRACT

**INTRODUCTION:** Glucose transporter type 1 deficiency syndrome is a metabolic encephalopathy that results from impaired glucose transport into the brain as the result of a mutation of the *SLC2A1* gene. It has been recognized recently that these patients can present with a much broader clinical spectrum than previously thought. We describe a 3-year-old boy presenting with episodic ataxia. **CASE REPORT:** Our patient exhibited periodic abnormal eye movements, including opsoclonus, since he was 4 months of age. At 2 years of age, he experienced acute cerebellar ataxia after a vaccination. Since then, he has had periodic attacks of ataxic gait, repeated vomiting, and abnormal eye movement. He was diagnosed as having episodic ataxia type 2 because the administration of acetazolamide seemed effective. By 3 years and 10 months of age, he exhibited mild mental retardation and mild trunk ataxia. The attacks were more likely to occur when he was hungry. Molecular analysis revealed that the *SLC2A1* gene had a de novo mutation of heterozygous seven nucleotide insertion within exon 7, resulting in a frameshift. He has recently begun a modified Atkins diet; the frequency of attacks has been reduced, and his psychomotor and language skills have begun to develop. **DISCUSSION:** Glucose transporter type 1 deficiency syndrome should be considered in the differential diagnosis in children with episodic ataxia, even if acetazolamide is effective.

***Keywords:*** glucose transporter type 1 deficiency syndrome, episodic ataxia, *SLC2A1*, cerebellar ataxia, intellectual disability, seizure, ketogenic diet

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## Introduction

Glucose transporter type 1 deficiency syndrome (GLUT1-DS) is caused by a defect of the glucose transporter, Glut1, the fundamental vehicle that facilitates glucose entry into the brain and across the astrocyte membrane, which is coded by the *SLC2A1* gene. GLUT1-DS was first described in 1991 as a metabolic encephalopathy characterized by epileptic seizures, delayed development, ataxia, dystonia, and acquired microcephaly (classic GLUT1-DS).[1]

The laboratory hallmark of GLUT1-DS is a low cerebrospinal fluid (CSF) glucose concentration (<40 mg/dL) and a low ratio of CSF/blood glucose (<0.4), Recently, a broader spectrum of complex clinical presentations of GLUT1-DS has been recognized.[2] Neurological features may be divided into three symptom domains: seizure, movement disorders, and cognitive/behavioral disturbances.[3] Three different phenotypes of GLUT1-DS are defined; (1) classical (early <2 years of age and late >2 years of age), (2) nonclassical, and (3) GLUT1-DS with minimal symptoms.[4] Here, we describe a 3-year-old boy with GLUT1-DS who presented with episodic ataxia.

## Case Report

This 3-year-old boy was the first child of healthy unrelated parents. He was born at 39 weeks of gestational age after an uneventful pregnancy and delivery. He had recurrent attacks of abnormal eye movement, including opsoclonus, at 4, 7, and 11 months of age. Epilepsy was initially suspected, but he did not receive antiepileptic treatment because the

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findings of an electroencephalography did not reveal epileptic discharges. He started walking independently at 18 months of age. When he was 2 years of age, he could not stand or walk for 2 weeks after receiving vaccinations for *Haemophilus influenzae* Type b, diphtheria, pertussis, and tetanus. He was diagnosed with acute cerebellar ataxia and recovered completely in a few days after hydration treatment. He was referred to our medical center at 2 years of age for the evaluation of his delayed development. He had no apparent neurological abnormalities, no pyramidal or extrapyramidal signs, and no cerebellar signs, such as ataxic gait or abnormal eye movement. He started to use meaningful words at 2 years of age, and his receptive language skills seemed better than his expressive language skills. He was diagnosed with a mild intellectual disability or delayed expressive language disorder.

Beginning at 2 years and 4 months of age, he had recurrent attacks of acute cerebellar ataxia that lasted for 2-3 hours, once or twice a month. The attacks consisted of three features: ataxic gait, repeated vomiting, and abnormal eye movement. Because oral acetazolamide treatment reduced the frequency of the attacks, episodic ataxia type 2 (EA2) was suspected. Magnetic resonance imaging of his brain did not show cerebellar atrophy or other abnormalities. When he was 3 years of age, his mother noticed that the attacks were more likely to occur when he was hungry, mostly in the early morning, and that his intermittent ataxic gait improved after having a meal, especially greasy food such as fried chicken. He had his first afebrile generalized tonic seizure at 3 years of age, and the findings of electroencephalography revealed no paroxysmal discharges.

At 3 years and 10 months of age, he was admitted for evaluation. His height, weight, and head circumference were 97 cm (−0.7 SD), 14.3 kg (−0.6 SD), and 47.7 cm (−1.6 SD), respectively, indicating acquired microcephaly. A neurological examination revealed mild truncal ataxia but no pyramidal or extrapyramidal signs. His developmental quotient was 47, indicating moderate intellectual disability. CSF glucose was 38 mg/dL and blood glucose was 102 mg/dL, with a CSF/blood ratio of 0.37. Levels of CSF lactate and pyruvate were normal (0.90 and 0.065 mmol/L, respectively). Thus, GLUT1-DS was suspected. Analysis of the DNA extracted from his blood revealed that *SLC2A1* has a de novo and novel heterozygous mutation of seven nucleotides insertion in exon 7, resulting in truncated protein [c.930_931 ins-GGATACC, p.Ile311 fs], and his diagnosis was confirmed.

He has recently begun a modified Atkins diet, eating five times per day. The frequency of his attacks is reducing, his psychomotor and language skills are improving, and he can now speak in multiple sentences and can concentrate.

## Discussion

The phenotypic spectrum of GLUT1-DS appears to be more variable than previously recognized. Our patient presented with intermittent cerebellar ataxia as the initial manifestation, similar to EA2, with mild truncal ataxia, whereas dystonia, chorea, or epilepsy were not apparent and cognitive disturbance was mild to moderate. Only one patient has been reported to have had intermittent ataxia as the initial manifestation and used acetazolamide like our patient.[5] In some reports, authors describe patients with cerebellar ataxia, but most of their cerebellar ataxia is chronic, and most had other motor abnormalities, including abnormal gait, dystonia, chorea, cerebellar intention tremor, and myoclonus.[4]

It is important to consider that GLUT1-DS and other channelopathies, such as EA2 caused by mutations of the *CACNA1A* gene, share chronic and intermittent clinical features and responsiveness to acetazolamide.[6] It is possible that chronic neuroglycopenia may lead to developmental alternations in channel expression or function, causing abnormal neuronal excitability in GLUT1-DS.[7] Responsiveness to acetazolamide may be a key component in the diagnosis of GLUT1-DS.[8]

A relationship between the clinical severity and the specific type of *SLC2A1* mutation has been noted.[4] Considering that many patients with atypical or milder cases remain to be diagnosed, it seems too hasty to establish the genotype–phenotype correlations. As discussed in the paragraphs to follow, in addition to the type of mutation, the patient's dietary habit from infancy may affect the phenotype.

This finding provides a clue in the diagnosis of GLUT1-DS in terms of confirming the correlation between fluctuations of neurological symptoms and fasting. Some patients have characteristic dietary habits that prevent a deterioration of neurological functioning. Some patients eat meals every 2-3 hours and wake at night to eat sweets. Another patient preferred to be served honey at bedside before rising in the morning.[9]

A ketogenic diet is currently the treatment of choice for GLUT1-DS. A ketogenic diet markedly improves seizures, movement disorder, and head growth. Recently, a modified Atkins diet has also been used successfully in patients with GLUT1-DS.[10] Our patient has recently commenced dietary treatment on the basis of a modified Atkins diet. The frequency of attacks is reduced, and his psychomotor and language skills have begun developing, although longer observation is needed to confirm that this therapy is effective. We should consider GLUT1-DS as a differential diagnosis in pediatric patients with intermittent cerebellar ataxia such as EA2 or intermittent neurological phenotypes such as other channelopathies.

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