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Region-specific impairments in striatal synaptic transmission and impaired instrumental learning in a mouse model of Angelman syndrome

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Abstract

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by mental retardation and impaired speech. Because patients with this disorder often exhibit motor tremor and stereotypical behaviors, which are associated with basal ganglia pathology, we hypothesized that AS is accompanied by abnormal functioning of the striatum, the input nucleus of the basal ganglia. Using mutant mice with maternal deficiency of AS E6-AP ubiquitin protein ligase Ube3a (*Ube3a*^{m-/p+}), we assessed the effects of *Ube3a* deficiency on instrumental conditioning, a striatum-dependent task. We used whole-cell patch-clamp recording to measure glutamatergic transmission in the dorsomedial striatum (DMS) and dorsolateral striatum (DLS). *Ube3a*^{m-/p+} mice were severely impaired in initial acquisition of lever pressing. Whereas the lever pressing of wild-type controls was reduced by outcome devaluation and instrumental contingency reversal, the performance of *Ube3a*^{m-/p+} mice were more habitual, impervious to changes in outcome value and action–outcome contingency. In the DMS, but not the DLS, *Ube3a*^{m-/p+} mice showed reduced amplitude and frequency of miniature excitatory postsynaptic currents. These results show for the first time a selective deficit in instrumental conditioning in the *Ube3a* deficient mouse model, and suggest a specific impairment in glutamatergic transmission in the associative corticostriatal circuit in AS.

Keywords

Angelman's syndrome; habit; instrumental conditioning; learning; striatum; UBE3A

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Introduction

Angelman syndrome (AS) is a neurodevelopmental disorder associated with profound cognitive disability, seizures, absence of speech, and movement disorders. A loss-of-function mutation in the maternal allele of the brain-specific *UBE3A* gene is responsible for the major clinical symptoms. The product of the *UBE3A* gene is an ubiquitin ligase enzyme widely expressed in the brain (Gustin *et al.*, 2010). Localized at synapses, Ube3a is thought to play a role in synapse development and plasticity in the brain (Jiang *et al.*, 1998; Dindot *et al.*, 2008; Yashiro *et al.*, 2009; Sato & Stryker, 2010).

Mouse models of AS have been generated, using selective genetic deletion of the *Ube3a* gene (Jiang *et al.*, 1998, 2010; Miura *et al.*, 2002). Mice inheriting the *Ube3a* mutation maternally have behavioral features commonly associated with AS, including susceptibility to seizures and deficits in learning and motor coordination (Jiang *et al.*, 1998; Mabb *et al.*, 2011). Ube3a deficiency is associated with impaired long-term potentiation at the hippocampal Schaffer collateral synapse, and increased levels of phospho-calcium/calmodulin-dependent protein kinase II (CaMKII) in the hippocampus (Weeber *et al.*, 2003). Behavioral tests also revealed a deficit in contextual fear conditioning (Jiang *et al.*, 1998), yet hippocampal lesions typically produce amnesia without significant motor or speech impairments (Squire *et al.*, 1993). Patients with hippocampal damage and AS patients thus do not show similar symptoms.

On the other hand, the major symptoms associated with AS, i.e. tremor, stereotypical behavior, gait disturbance and speech impairment, are usually associated with dysfunction of the basal ganglia, a set of subcortical nuclei known to be critical for learning and generation of voluntary actions (Alexander & Crutcher, 1990). Previous work also found altered dopamine levels in mouse models of AS (Farook *et al.*, 2012), suggesting changes in the projections from the midbrain dopamine neurons to their targets in the basal ganglia. In addition, *Ube3a*^{m-/p+} mice also displayed significant deficits on the rotarod, a task that has been used to study basal ganglia function, in particular striatal plasticity (Costa *et al.*, 2004; Yin *et al.*, 2009).

Based on these observations, we hypothesize that the clinical symptoms in AS can be attributed to defects in the basal ganglia, in particular the striatum, the major input nucleus, which has been shown by previous work to be critical for instrumental learning, the learning of the relationship between action and outcomes (Graybiel, 1998; Yin *et al.*, 2005a,b; Yin & Knowlton, 2006). The major symptoms of AS, such as mental retardation and impairments in speech and motor control, may be due to a basic deficit in striatum-dependent instrumental learning, which is required for learning to generate appropriate behaviors to reach specific goals in all organisms. We therefore tested the effect of Ube3a deficiency on instrumental learning using a mouse model (Jiang *et al.*, 1998). We found a striking deficit in the initial acquisition of novel actions as well as impaired goal-directed control of behavior.

Using whole-cell patch-clamp recording, we also assessed glutamatergic transmission in the striatum in adult mice with *Ube3a*^{m-/p+} and their wild-type littermates. We found that *Ube3a* deficiency selectively reduced glutamatergic transmission in the dorsomedial striatum

(DMS; associative striatum), a region that has been previously shown to be critical in the acquisition of goal-directed actions in instrumental conditioning (Yin *et al.*, 2005a,b).

Materials and methods

Ube3a-mutant mice

All procedural guidelines are in accordance with the Guidelines laid down by the National Institutes of Health regarding the care and use of animals. This study received institutional approval from Duke University Animal Care and Use Committee (protocol A0163-13-06). Mice with a null mutation for *Ube3a* have been reported previously (Jiang *et al.*, 1998). Maternal deficiency heterozygotes on the C57BL6J background (> F10 backcrossing to C57BL6J) were used (*Ube3a*^{m-/p+}), and wild-type littermates (*Ube3a*^{m+/p+}) were used as controls. The genotyping protocol has been described previously (Jiang *et al.*, 1998).

Instrumental conditioning

The basic procedure for instrumental training in mice has been described previously (Yu *et al.*, 2009; Rossi & Yin, 2012; Rossi *et al.*, 2012). Briefly, training took place in Med Associates (St Albans, VT, USA) mouse operant chambers. Each chamber has a food magazine that receives Bio-Serv 14-mg pellets from a dispenser, two retractable levers on either side of the magazine, and a 3-W 24-V house light mounted on the wall opposite the levers and magazine. A computer with the Med-PC-IV program was used to control the chambers and record behavior. For the behavioral experiments, seven *Ube3a*^{m+/p+} and seven *Ube3a*^{m-/p+} naïve male mice (2–4 months old) were used. All mice were food-deprived to ~85% of their *ad libitum* body weights before the start of behavioral experiments.

At the beginning of each session, the house light was turned on and the lever inserted. At the end of each session, the house light was turned off and the lever was retracted. Initial lever-press training consisted of five consecutive days of fixed-ratio 1 (FR1 or continuous reinforcement), during which the animals received a pellet for each lever press. The FR1 sessions ended after 1 h or 30 rewards. Following FR1 training, mice were trained on FR5 (pellet delivered after five lever presses) for 2 days, FR10 for 1 day and FR20 for 5 days. Each of these sessions ended after 1 h. Ratio schedules of reinforcement were used because these schedules are known to generate goal-directed actions, the performance of which is reduced by outcome devaluation (Dawson & Dickinson, 1990).

Outcome devaluation

We used pre-feeding to reduce the value of the outcome. Rather than the specific satiety (reducing the incentive value of the specific food reward but controlling for the overall level of satiety), we used the strongest form of devaluation by simply inducing satiety using the food reward earned by lever pressing during training.

Each mouse was left in a clean cage with unlimited access to food pellets. The pre-feeding session lasted ~60–90 min, during which each mouse in the devalued group consumed at least 0.6 g of the pellets. Immediately following pre-feeding, the mice were returned to the instrumental chambers for a 5-min probe session conducted in extinction. Each animal was

tested once a day for 2 days. Four of the mice from each group were devalued on the first day, and the rest were not. On the second day, those mice in the devalued group on the first day were not devalued, and the other mice received the pre-feeding treatment. A within-subject comparison between devalued and valued conditions reveals whether performance was altered by the outcome devaluation. To ensure that the level of motivation was not different between groups, we also measured the amount of food consumed during the pre-feeding session.

Omission

Following outcome devaluation, the mice were retrained for two more sessions at FR20, followed by two sessions of omission training, which is a behavioral assay designed to assess the persistence and inflexibility of acquired instrumental behavior. The imposition of the omission contingency reverses the previously learned relationship between action and outcome. A pellet was delivered every 20 s without lever pressing, but each press would reset the counter and thus delay the food delivery. Thus pressing cancels the reward whereas not pressing leads to reward delivery. Insensitivity to such a reversal of the instrumental contingency indicates compulsive and inflexible behavioral control.

Whole-cell patch-clamp electrophysiology

For whole-cell patch-clamp recordings, coronal slices were cut from adult *Ube3a*^{m-/p+} mice ($n = 14$) between 2 and 4 months of age and their wild-type littermates (*Ube3a*^{m+/p+}; $n = 13$). Both males and females were used. After acute isoflurane anesthesia, the mice were decapitated and their brains removed and placed in ice-cold cutting solution bubbled with 95% O₂ and 5% CO₂ containing the following (in mM): sucrose, 194; NaCl, 30; KCl, 2.5; MgCl₂, 1; NaHCO₃, 26; NaH₂PO₄, 1.2; and D-glucose, 10. After 5 min, 250- μ m slices were cut using a Vibratome 1000 brain slicer. Coronal slices were cut for evoked excitatory postsynaptic current (EPSC) experiments. To preserve as much of the glutamatergic projections as possible, for miniature EPSC (mEPSC) experiments sagittal slices were used.

During the recovery period slices were placed in 35°C oxygenated aCSF solution containing the following (in mM): NaCl, 124; KCl, 2.5; CaCl₂, 2; MgCl₂, 1; NaHCO₃, 26; NaH₂PO₄, 1.2; and D-glucose, 10. All recordings were made under continuous perfusion of aCSF with 2–3 mL/min flow rate. Pipettes (2.5–5 M Ω) for voltage-clamp experiments contained the following (in mM): cesium methane sulfonate, 120; NaCl, 5; tetraethylammonium chloride, 10; HEPES, 10; lidocaine *N*-ethyl bromide, 4; EGTA, 1.1; magnesium ATP, 4; and sodium GTP, 0.3; pH adjusted to 7.2 with CsOH and osmolarity set to 298 mOsm with sucrose. For voltage-clamp recordings, picrotoxin (50 μ M) was added to the bath to block GABAergic transmission. All chemicals were purchased from Sigma or Tocris.

To measure neuronal excitability, current-clamp recording was performed from coronal mouse brain slices as previously described (Rossi *et al.*, 2012). Internal solution for current-clamp experiments contained (in mM): potassium gluconate, 150; MgCl₂, 2; EGTA, 1.1; HEPES, 10; sodium ATP, 3; and sodium GTP, 0.2; with pH adjusted to 7.2 with KOH and osmolarity set to ~300 mosM with sucrose.

All recordings were performed with an Axopatch 1D amplifier (Axon Instruments). The data were filtered at 5 kHz and digitized at 10 kHz. Slices were maintained between 28 and 30°C during the experiment. We recorded from medium spiny projection neurons in the dorsolateral striatum (DLS; sensorimotor striatum) and DMS (associative striatum), visually guided with the aid of differential interference contrast. We analyzed only recordings with series resistance < 20 MΩ.

EPSC amplitudes were examined using peak detection software in pCLAMP10 (Molecular Devices). In voltage-clamp mode (cells held at −70 mV), we recorded mEPSCs with 1 μM tetrodotoxin (TTX) in the bath solution. For evoked EPSCs, test stimuli were delivered via a Master-8 stimulator (A.M.P.I., Jerusalem, Israel) at a frequency of 0.05 Hz through a bipolar twisted tungsten wire placed in the striatum. The duration and intensity of the stimulation were adjusted so that the amplitude of the evoked EPSC was 200–500 pA. To record the NMDA component of the current, the cell was voltage-clamped at 40 mV, and the peak amplitude 50 ms after the stimulus artifact was used as the measure of the slow NMDA component. To obtain the NMDA/AMPA ratio, the NMDA component was divided by absolute peak amplitude of the evoked current measured at −70 mV (AMPA component).

Data analysis

Data from whole-cell patch-clamp recordings were analyzed using pCLAMP10 (Molecular Devices). Behavioral data were analyzed using Microsoft Excel and Graphpad Prism. All statistical analyses (ANOVAS and *t*-tests) were performed using Graphpad Prism, and a *P*-value of 0.05 was used as a threshold for statistical significance.

Results

Lever press acquisition and performance

All mice learned to press the lever after five sessions of FR1 training (continuous reinforcement), in which each press earned a food pellet. However, compared to the controls, the *Ube3a*^{m-/p+} mice were significantly impaired in acquisition (Fig. 1A). A two-way mixed ANOVA conducted on the initial phase of lever press acquisition, with Days and Genotype as factors, showed a main effect of Genotype ($F_{1,65} = 33.0$, $P < 0.0001$), a main effect of Days ($F_{4,65} = 7.4$, $P < 0.0001$), and no interaction between these factors ($F_{4,60} = 2.1$, $P > 0.05$).

The mice were then progressively trained on FR5, FR10 and FR20 schedules. Eventually, however, all mice reached similar rates of lever pressing, as shown by performance on the FR20 schedule (Fig. 1B). A two-way mixed ANOVA conducted on lever-press rates from the last 5 days of training revealed no main effect of Genotype ($F_{1,65} < 1$, $P > 0.05$), no main effect of Days ($F_{4,65} < 1$, $P > 0.05$), and no interaction between these factors ($F_{4,60} < 1$, $P > 0.05$).

Devaluation

As shown in Fig. 1C, the lever pressing of *Ube3a*^{m-/p+} mice was less sensitive to changes in outcome value. Planned comparison between the devalued and valued conditions for each mouse showed that the wild-type controls pressed more when the outcome was valued ($P <$

0.05). Their performance was thus adjusted according to the expected value of the outcome. By contrast, the lever pressing of *Ube3a*^{m-/p+} mice was not significantly different between the valued and devalued conditions ($P > 0.05$). This result cannot be explained by different amounts of food consumed during the pre-feeding session, as there was no significant group difference in food consumed between ($P > 0.05$; Fig. 1C).

Omission

The reversal of the instrumental contingency produced different effects on *Ube3a*^{m-/p+} and *Ube3a*^{m+/p+} mice (Fig. 1D). This observation was confirmed by a mixed two-way ANOVA with Session and Genotype as factors: there was a main effect of Session ($F_{1,12} = 4.8$, $P < 0.05$), no main effect of Genotype ($F_{1,12} < 1$, $P > 0.05$), and a significant interaction between these two factors ($F_{1,12} = 7.5$, $P < 0.05$). *Post hoc* analysis revealed that there was no difference between the two groups during session 1 ($P > 0.05$), but *Ube3a*^{m-/p+} mice showed higher rates of pressing during the second session ($P < 0.01$).

Whole-cell patch-clamp striatal recording in brain slices

Previous work has established the DMS as critical for instrumental learning (Yin *et al.*, 2005a,b; Hilario *et al.*, 2012). The behavioral results resemble the effects of DMS lesions. To study the effects of *Ube3a* deficiency on synaptic transmission in the DMS, we performed whole-cell patch-clamp recording from medium spiny projection neurons in the DMS and DLS, and compared striatal mEPSCs from *Ube3a*^{m-/p+} mice and wild-type littermates.

Compared to the wild-type controls, *Ube3a*^{m-/p+} mice showed significant differences in synaptic transmission. In the DMS, the frequency and amplitude of mEPSCs were reduced in *Ube3a*^{m-/p+} mice (Fig. 2; $P = 0.002$ for amplitude, $P = 0.02$ for frequency). This pattern was not observed in the DLS (Fig. 3; all $P > 0.05$ for amplitude and frequency). These results suggest that basal synaptic transmission was selectively impaired in the DMS but not in the DLS.

As shown in Fig. 4, the paired-pulse ratio (PPR; second EPSC divided by first EPSC evoked by two pulses 50 ms apart; data not shown), which is inversely correlated with the probability of presynaptic glutamate release, was not different between the two groups in either DLS or DMS (DLS: *Ube3a*^{m+/p+} PPR = 1.58 ± 0.06 , $n = 17$; *Ube3a*^{m-/p+} PPR = 1.45 ± 0.07 , $n = 18$; $P > 0.05$; DMS: *Ube3a*^{m+/p+} PPR = 1.44 ± 0.06 , $n = 16$; *Ube3a*^{m-/p+} PPR = 1.39 ± 0.07 , $n = 15$; $P > 0.05$, unpaired *t*-test). There was also no significant difference between wild-type and *Ube3a*^{m-/p+} in the NMDA/AMPA ratio in DLS or DMS (*t*-test, all $P > 0.05$; Fig. 4A).

Finally, we also characterized the intrinsic properties of medium spiny projection neurons, including resting membrane potential and response to current injection. There was no significant difference in resting membrane potential between controls and *Ube3a*^{m-/p+} in either the DMS or the DLS (*t*-test, all $P > 0.05$; Fig. 4B) but we found reduced excitability in the DLS (two-way ANOVA with Current and Genotype as factors; main effect of Genotype, $F_{1,144} = 11.9$, $P < 0.01$; main effect of Current, $F_{3,144} = 58.8$, $P < 0.001$; and a significant interaction between these factors; $F_{3,144} = 9.8$, $P < 0.0001$; *post hoc* analysis revealed higher

spike frequency in *Ube3a^{m-/p+}* mice when the current injected was 300 pA or 400 pA, all $P < 0.001$). By contrast, excitability was not altered in the DMS in the *Ube3a m-/p+* mice (no main effect of Genotype, $F_{1,84} < 1$, $P > 0.05$; a main effect of Current, $F_{1,84} = 14.7$, $P < 0.0001$, and no significant interaction between these factors, $F_{3,84} = 1.1$, $P > 0.05$).

Discussion

We hypothesized that the major symptoms of AS are due to impaired striatal functioning. To test this hypothesis, we first assessed the effect of Ube3a deficiency on striatum-dependent instrumental learning (Yin & Knowlton, 2006). We found that genetic deletion of the *Ube3a* gene produced a major deficit in the initial learning of instrumental actions. *Ube3a^{m-/p+}* mice were impaired at learning the relationship between actions and outcomes. As revealed by devaluation and omission, tests of behavioral persistence and compulsivity, the behavior of *Ube3a^{m-/p+}* mice was more impervious to changes in outcome value and action–outcome contingency (Fig. 1C and D). These results suggest that Ube3a is critical for goal-directed behavioral control, a major measure of intelligence and behavioral flexibility (Balleine & Dickinson, 1998). Because *Ube3a^{m-/p+}* mice were able to press the lever just as quickly as controls, the observed effects cannot be attributed to a general motor deficit in these animals, nor did they show any clear abnormalities in motivation for food, as the amount of food they consumed during the prefeeding session was similar to that of controls (Fig. 1C).

Insensitivity to omission may indicate Pavlovian control over lever-pressing behavior (Schwartz & Gamzu, 1977). But this is also a characteristic of habitual behavior, as shown by previous studies (Dickinson *et al.*, 1998; Yin *et al.*, 2006a; Yu *et al.*, 2009). By itself the omission test may not be sufficient to establish habitual behavior, but the combination of devaluation and omission tests, as used in this study, can ascertain that the lever pressing of *Ube3a^{m-/p+}* mice is similar to the habitual lever pressing observed in many previous studies.

In particular, the pattern of behavioral deficits observed in *Ube3a^{m-/p+}* mice is similar to that observed following lesion or inactivation of the associative striatum (DMS), which raises the possibility that *Ube3a^{m-/p+}* mice have a selective deficit in synaptic transmission in the DMS. This prediction was confirmed by whole-cell patch-clamp recording experiments. Compared to the wild-type controls, excitatory glutamatergic transmission in *Ube3a^{m-/p+}* mice was reduced in the DMS, but not in the DLS, in accord with previous work showing a functional dissociation between these regions in instrumental learning and behavior (Yin *et al.*, 2004, 2005b; Yin, 2010). Together these results revealed a key role of the basal ganglia in the learning and behavioral deficits of AS, and shed light on the cellular and circuit mechanisms underlying the deficits in learning and behavior.

One alternative explanation of our results should be considered. Because our instrumental training sessions are limited by time, it is possible that the *Ube3a^{m-/p+}* mice had less experience with the action–outcome contingency, because their rate of lever pressing was lower, especially early in training (Fig. 1). Thus reduced learning experience may explain the observed deficits. This possibility, however, does not affect our interpretation of the data. In fact, it provides even stronger support for the hypothesis that Ube3a deficiency impairs learning of instrumental contingencies. It is well established that sensitivity to devaluation

and omission is reduced with extensive training (Adams, 1982; Dickinson, 1994; Derusso *et al.*, 2010). If the *Ube3a*^{m-/p+} mice received less exposure to the instrumental contingency because they pressed the lever less often then they should be expected to show more sensitivity to devaluation and omission. However, the opposite is true: despite receiving slightly less training, the performance of *Ube3a*^{m-/p+} mice was still more habitual than that of the controls.

Previous work found that *Ube3a*^{m-/p+} mice showed impaired hippocampal LTP and contextual fear conditioning (Jiang *et al.*, 1998). Although the hippocampal deficits may account for some of the learning deficits in AS, most of the major symptoms are clearly distinct from those observed in patients or animals with hippocampal pathology (Squire *et al.*, 1993). Compared to fear conditioning, instrumental conditioning, the association of actions with their consequences, is a more general type of learning, relevant for the acquisition of all new actions. Thus the striking deficits observed in *Ube3a*^{m-/p+} mice may be more closely related to the deficits in speech and mental retardation in human AS patients.

The deficits in initial acquisition of lever pressing (Fig. 1A) in *Ube3a*^{m-/p+} mice suggest reduced excitatory inputs to the DMS from association cortical areas and intralaminar thalamus but it is not clear whether the cortical and thalamic glutamatergic inputs are differentially affected, nor is it known whether and which of the association cortical regions projecting to the DMS show some form of pathology. Our results certainly do not exclude abnormalities upstream in cortical and thalamic areas that project to the striatum. For example, the prefrontal cortex and mediodorsal thalamus also play important roles in instrumental learning (Corbit & Balleine, 2003; Corbit *et al.*, 2003; Yu *et al.*, 2010, 2012). Future studies will be needed to examine the function of these regions in *Ube3a*^{m-/p+} mice.

Previous work has established a functional dissociation between DMS and DLS in the learning and control of instrumental behavior, the acquisition of new behavioral patterns in the service of a goal (Yin & Knowlton, 2004; Yin *et al.*, 2004, 2005a,b, 2006a, 2009; Corbit & Janak, 2010; Thorn *et al.*, 2010; Yin, 2010). The DMS (associative striatum) receives strong projections from the association cortices such as the prefrontal cortex (McGeorge & Faull, 1989). It is critical for initial acquisition of the action–outcome contingency, the basic feedback function in instrumental conditioning (Balleine *et al.*, 2007). On the other hand, the DLS (sensorimotor striatum) receives inputs from the primary sensorimotor cortices. It is implicated in habitual control of behavior (Yin & Knowlton, 2006) and in the formation of more automatic behavioral sequences (Cromwell & Berridge, 1996; Graybiel, 1998; Yin, 2010; Dezfouli & Balleine, 2012).

What is responsible for the regional differences in striatal transmission? Assuming the imprinting expression is similar in DLS and DMS, one hypothesis is that the interaction between *Ube3a* and different proteins may differ from region to region. DLS and DMS show significant differences in synaptic transmission and plasticity as a result of the gradients in the expression of various receptors (Gerdeman *et al.*, 2003; Dang *et al.*, 2006; Yin *et al.*, 2007, 2009), e.g. the expression of the CB1 endocannabinoid receptors is higher in the DLS than in the DMS (Herkenham *et al.*, 1991; Gerdeman *et al.*, 2003). DMS and DLS also differ

in their dopaminergic innervation (Joel & Weiner, 2000). DA neurons from the medial midbrain project to more medial striatal regions whereas DA neurons from the lateral midbrain project to more lateral striatal regions. The mechanisms of dopamine uptake and binding to dopamine receptors can also vary across striatal regions (Cragg *et al.*, 2000; Yin *et al.*, 2009). Interestingly, the motor impairments in AS, in particular tremor, can be ameliorated by Levodopa treatment, the most common dopamine replacement therapy used to treat Parkinson's disease. Such observations suggest that AS may also involve reduced dopaminergic signaling in the striatum, and this has been confirmed by direct measurement of DA using fast-scan cyclic voltammetry (Riday *et al.*, 2012). Given the well-established dopamine–glutamate interactions in the striatum (Surmeier *et al.*, 2007), this mechanism could be involved in the changes in glutamatergic transmission observed here (Joel & Weiner, 2000; Voorn *et al.*, 2004).

Our results suggest for the first time that glutamatergic transmission in the DMS may play a role in AS pathogenesis. The region-specific changes in synaptic transmission we observed may be responsible for the major learning and motor symptoms of AS. Although our study merely represents a first step towards elucidating the involvement of the basal ganglia in this disorder, it opens up a new avenue of research on AS. For example, it would be important to elucidate the effects of *Ube3a* deficiency in the development of cortex–basal ganglia circuits and in long-term plasticity in the striatum (Gerdeman *et al.*, 2002; Kreitzer & Malenka, 2005; Yin *et al.*, 2006b; Adermark & Lovinger, 2007; Shen *et al.*, 2008). Studies will also be needed to assess the relative involvement of specific striatal cell populations (e.g. striatopallidal vs. striatonigral, striosome vs. matrix) in AS, as well as that of the corticostriatal and thalamostriatal projections (Ding *et al.*, 2008; Yin *et al.*, 2009). Finally, *in vivo* measures of striatal activity will be needed to elucidate the consequences of the synaptic deficits are for neural activity in the striatum during behavior.

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Abbreviations

AS	Angelman syndrome
CaMKII	phospho-calcium/calmodulin-dependent protein kinase II
DLS	dorsolateral striatum
DMS	dorsomedial striatum
EPSC	excitatory postsynaptic current
FR1	fixed-ratio 1
FR5	pellet delivered after five lever presses
mEPSC	miniature EPSC

PPR	paired-pulse ratio
TTX	tetrodotoxin.

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Fig. 1.

Impaired instrumental learning and loss of goal-directed instrumental control in *Ube3a^{m-/p+}* mice. (A) *Ube3a^{m-/p+}* mice were significantly impaired during initial acquisition of an instrumental action. Shown are rates of lever pressing during the first 5 days of instrumental training on an FR1 (continuous reinforcement) schedule. Each lever press was followed by a food pellet. (B) Additional training on leaner FR schedules showed that *Ube3a^{m-/p+}* mice eventually learned to press the lever as well as the wild-type controls. On the FR20 schedule, they reached press rates that were similar to those of controls. (C) Consumption of food pellets during the pre-feeding session did not differ between genotypes but lever pressing during the outcome devaluation test (5 min, conducted in extinction) revealed a key difference. Lever press of *Ube3a^{m-/p+}* mice was reduced by outcome devaluation. (D) Omission test. The action–outcome contingency was reversed by presenting free rewards in the absence of lever pressing, but each time the lever was pressed the free reward was

delayed. Ube3a^{m-/p+} mice persisted in pressing the lever despite the imposition of the omission contingency.

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Fig. 2. *Ube3a^{m-p/+}* mice showed impaired glutamatergic synaptic transmission in DMS. (A) Illustration of the sagittal slices used to record from DMS and representative mEPSC traces. (B) Left, cumulative distribution of mEPSC amplitudes in the DMS. Right, mean mEPSC amplitude. *Ube3a^{m-p/+}* mice showed lower mEPSC amplitudes than did wild-type littermate controls ($P = 0.002$). $*P < 0.05$. (C) Left, cumulative distribution of intervals between mEPSCs. Right, mean mEPSCs frequency. *Ube3a^{m-p/+}* mice showed lower frequency than wild-type littermate controls ($P = 0.02$). $*P < 0.05$.

Fig. 3. *Ube3a^{m-p+}* mice did not show impaired glutamatergic synaptic transmission in DLS. (A) Illustration of the sagittal slices used to record mEPSCs from DLS and representative mEPSC traces. (B) Left, cumulative distribution of mEPSC amplitudes in the DLS. Right, mean mEPSC amplitude. There was no significant difference in mean mEPSC amplitude between *Ube3a^{m-p+}* mice and wild-type littermate controls ($P > 0.05$). (C) Left, cumulative distribution of intervals between mEPSCs. Right, mean mEPSCs frequency. There was no significant difference in mean mEPSC frequency between *Ube3a^{m-p+}* mice and wild-type littermate controls ($P > 0.05$).

Fig. 4.

Evoked EPSCs and intrinsic neuronal properties in *Ube3a^{m-p/+}* mice. (A) In the DLS, there was no difference between *Ube3a^{m-p/+}* mice and controls in PPR (amplitude of second EPSC divided by first EPSC, inter-stimulus interval = 50 ms), nor was there a difference in NMDA/AMPA ratio. (B) In the DLS, there was no group difference in resting membrane potential but neuronal excitability, as measured by spike frequency in response to current injection, was reduced in *Ube3a^{m-p/+}* mice. (C) In the DMS, there was no difference between *Ube3a^{m-p/+}* mice and controls in PPR or in NMDA/AMPA ratio. (D) In the DMS, there was no group difference in resting membrane potential or in neuronal excitability.