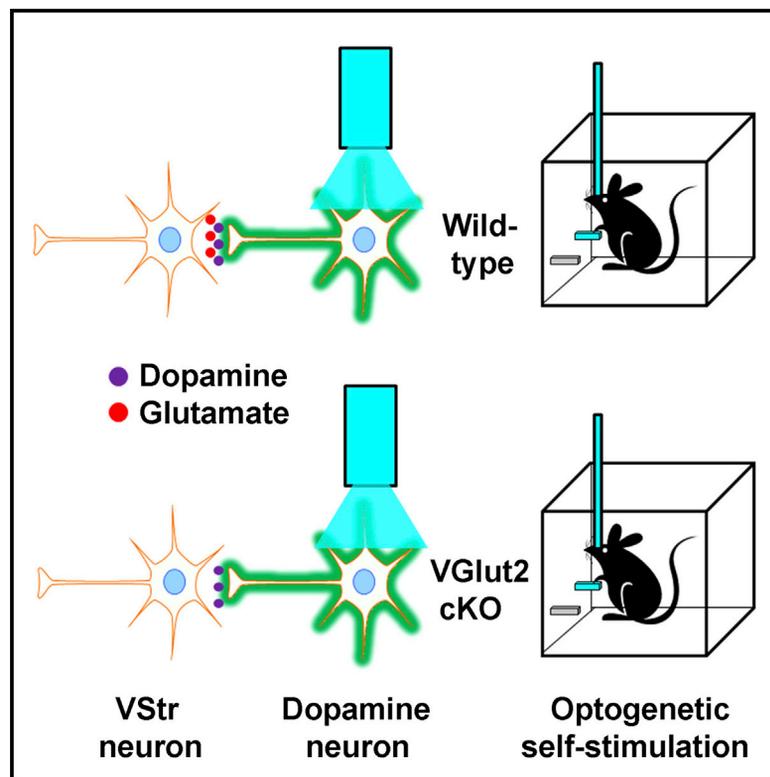


## Disrupting Glutamate Co-transmission Does Not Affect Acquisition of Conditioned Behavior Reinforced by Dopamine Neuron Activation

### Graphical Abstract



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### In Brief

Wang et al. find that targeted deletion of the gene encoding VGLUT2 in dopamine neurons largely eliminates fast excitatory responses caused by glutamate co-transmission in the ventral striatum in vivo. However, it has little effect on the acquisition of conditioned responses reinforced by the activation of VTA dopamine neurons.

### Highlights

- Glutamate co-release from DA neurons drives fast excitatory responses in VStr in vivo
- Loss of VGLUT2 in DA neurons abolishes glutamate co-transmission
- Such loss does not disrupt DA neurons from reinforcing behavior



# Disrupting Glutamate Co-transmission Does Not Affect Acquisition of Conditioned Behavior Reinforced by Dopamine Neuron Activation

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## SUMMARY

Dopamine neurons in the ventral tegmental area (VTA) were previously found to express vesicular glutamate transporter 2 (VGLUT2) and to co-transmit glutamate in the ventral striatum (VStr). This capacity may play an important role in reinforcement learning. Although it is known that activation of the VTA-VStr dopamine system readily reinforces behavior, little is known about the role of glutamate co-transmission in such reinforcement. By combining electrode recording and optogenetics, we found that stimulation of VTA dopamine neurons in vivo evoked fast excitatory responses in many VStr neurons of adult mice. Whereas conditional knockout of the gene encoding VGLUT2 in dopamine neurons largely eliminated fast excitatory responses, it had little effect on the acquisition of conditioned responses reinforced by dopamine neuron activation. Therefore, glutamate co-transmission appears dispensable for acquisition of conditioned responding reinforced by DA neuron activation.

## INTRODUCTION

Recent studies found that dopamine (DA) neurons in the ventral tegmental area (VTA) transmit both DA and glutamate (Chuhma et al., 2004, 2014; Dal Bo et al., 2004; Kawano et al., 2006; Koos et al., 2011; Morales and Root, 2014; Stuber et al., 2010; Sulzer et al., 1998; Tecuapetla et al., 2010; Trudeau et al., 2014; Zhang et al., 2015). The capacity of DA neurons to release glutamate and excite postsynaptic cells may play an important role in reinforcement learning, for which the activity of DA neurons is crucial. Phasic activity of DA neurons is said to indicate errors in predicting future reward, and such signals provide vital information in acquiring conditioned responding (Montague et al., 1996; Schultz et al., 1997). Indeed, optogenetic activation of the VTA-ventral striatum (VStr) DA system readily reinforces

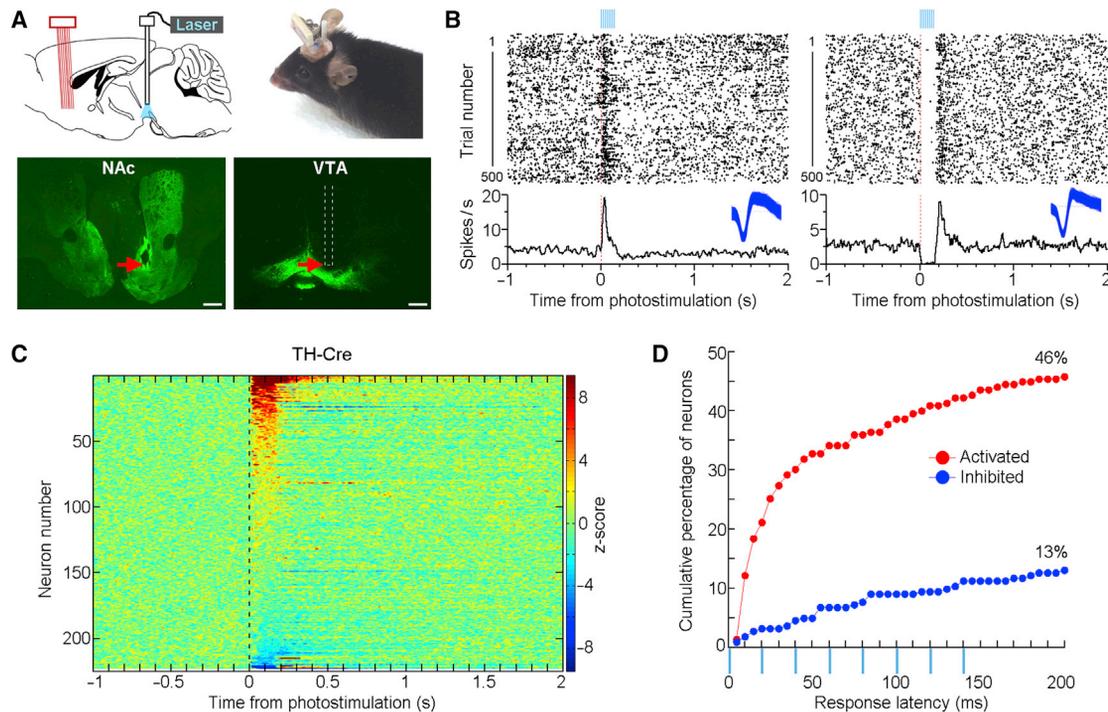
behavior that is contingently paired with such activation, thereby supporting intracranial self-stimulation (ICSS) (Ilango et al., 2014a; 2014b; Steinberg et al., 2014; Witten et al., 2011). Similarly, rodents learn to self-administer drugs that potentiate DA transmission such as cocaine and amphetamines both intravenously and intracranially, directly into the VStr; such self-administration is abolished by the administration of intra-VStr DA receptor antagonists or by 6-hydroxydopamine lesions (Ikemoto and Wise, 2004; Koob, 1992; Pierce and Kumaresan, 2006; Wise and Bozarth, 1987). However, little is known about the role of glutamate release from DA neurons in such reinforcement learning.

It is well known that the sooner a reward follows a behavior, the quicker the behavior becomes conditioned (Keeseey, 1964). Given that glutamate release excites post-synaptic neurons on the order of milliseconds, whereas DA transmission takes at least several tens of milliseconds, it is possible that the rapid action of glutamate co-transmission of DA neurons is important in acquiring reinforced behavior. The present study aims to test this by utilizing two separate conditional knockout mouse lines in which Slc17a6, the gene encoding vesicular glutamate transporter type 2 (VGLUT2), was deleted in DA neurons (Birgner et al., 2010; Hnasko et al., 2010). Our results show that optogenetic stimulation of VTA DA neurons indeed triggered fast excitatory responses in VStr neurons in vivo, and the conditional deletion of VGLUT2 diminished such excitatory responses. However, the conditional deletion of VGLUT2 had little or no effect on acquisition of conditioned responding reinforced with optogenetic stimulation of VTA DA neurons.

## RESULTS

### Optogenetic Stimulation of VTA DA Neurons Evokes Fast Phasic Activity in the VStr

We used VTA DA-specific optogenetic stimulation combined with VStr tetrode recording to examine how activation of VTA DA neurons affects single-neuron activity of VStr neurons in freely behaving TH-Cre mice (Figures 1A and S1). We delivered VTA optogenetic stimulation, using a parameter (a 50-Hz eight-pulse train; 8-mW intensity and 1-ms pulse width) known to



**Figure 1. Optogenetic Stimulation of VTA DA Neurons Evokes Fast Phasic Responses in the VStr of Freely Behaving TH-Cre Mice**

(A) Placements of an optic fiber above VTA DA neurons and an electrophysiological recording probe in the VStr. The red arrows in the bottom photomicrograms show a representative VTA stimulation site (right) and a VStr recording site (left). Scale bars, 0.5 mm. Green fluorescence indicates yellow fluorescent protein (YFP) expression.

(B) Peri-event rasters and histograms showing examples of VStr neurons excited (left) and inhibited (right) upon VTA optogenetic stimulation. Insets, 1,000 representative overlapping spike waveforms (blue traces; 1 ms) of the two neurons.

(C) Summary of the z-scored peri-event histogram of all the recorded VStr neurons ( $n = 223$ ) upon VTA optogenetic stimulation in TH-Cre mice ( $n = 8$ ). Peri-event histograms (bin size, 5 ms) were smoothed with a Gaussian filter (filter width, 3 bins).

(D) Cumulative percentage of the activated (red) and inhibited (blue) VStr neurons with response latencies of 200 ms or less.

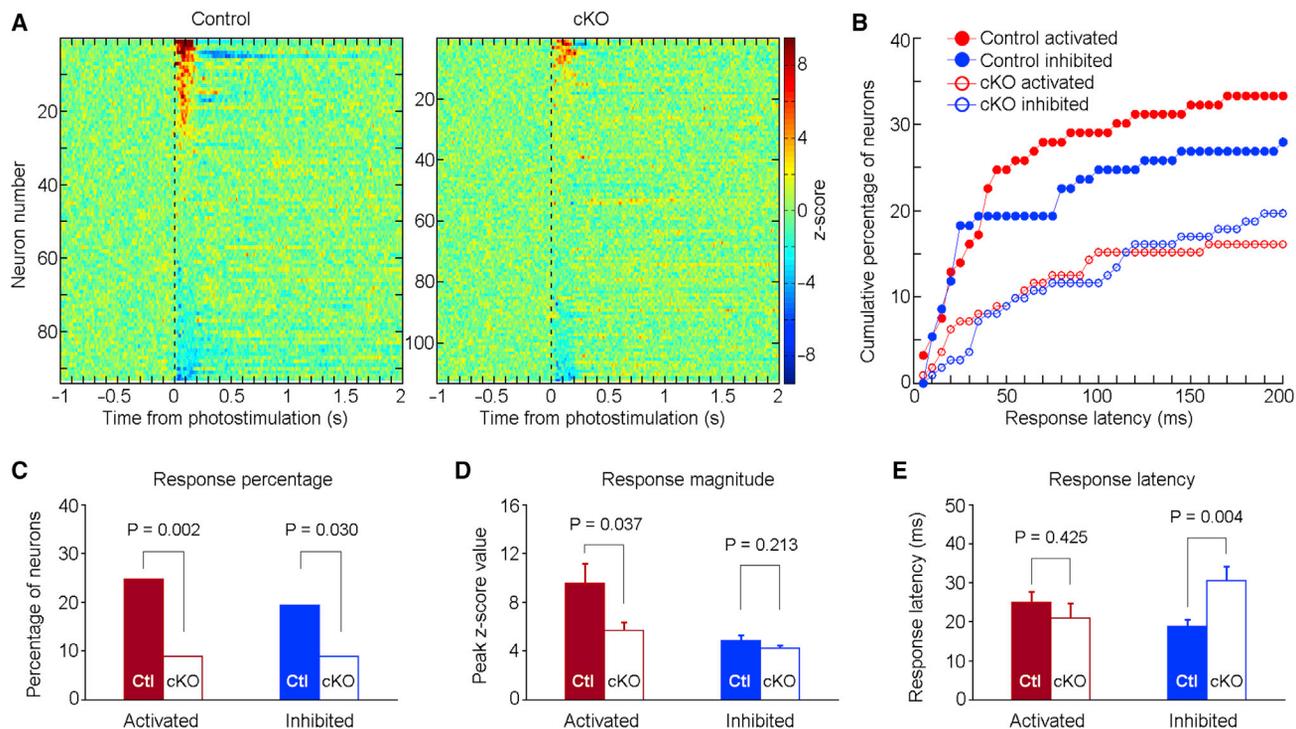
support ICSS (Ilango et al., 2014a), with variable intervals of 5–10 s (repeated 500 trials or more), while continuously recording VStr neural activity in freely behaving mice in their home cages. Many VStr neurons displayed fast excitatory or inhibitory responses to VTA optogenetic stimulation (Figures 1B, 1C, and S2). In total, 46% of the recorded VStr neurons displayed excitation within 200 ms upon VTA optogenetic stimulation, whereas a smaller percentage of VStr neurons (13%) displayed inhibition (Figure 1D). Moreover, most of these fast VStr responses evoked by VTA optogenetic stimulation were insensitive to DA D1 or D2 receptor antagonist (Figure S3).

**Conditional Knockout of VGLUT2 Attenuates VStr Fast Phasic Responses**

We hypothesized that fast activations, particularly those occurring within 50 ms upon VTA stimulation, are mediated by glutamate co-transmission of DA neurons. To address this, we used a mouse line with conditional knockout (cKO) of VGLUT2 ( $Slc17a6^{flox/flox}; Slc6a3^{+/IRESCre}$ ) in DA neurons (Birgner et al., 2010), which allowed selective activation of DA neurons without glutamate co-transmission. We injected a Cre-dependent adeno-associated virus (AAV)-ChR2 into the VTA of cKO and control littermates ( $Slc17a6^{+/flox}; Slc6a3^{+/IRESCre}$ ), and implanted

an optic fiber above the injection site. These mice were then tested behaviorally for acquisition of conditioned responding in an ICSS procedure, which we describe later, and thereafter a subset was further prepared to examine striatal neuron activity as a function of optogenetic stimulation of VTA DA neurons, which we describe immediately below.

We recorded 112 neurons from cKO ( $n = 6$ ) and 93 neurons from control mice ( $n = 6$ ) as a function of VTA stimulation (a 50-Hz eight-pulse train; 8-mW intensity and 1-ms pulse width) (Figure 2A). Twenty-five percent (23/93) of VStr neurons displayed excitatory responses within 50 ms, during which VStr neurons have likely not yet been affected by activities of G-protein-coupled DAergic receptors (Marcott et al., 2014). Only 9% (10/112) of the VStr neurons displayed such responses in cKO mice (Figures 2B and 2C). Moreover, the magnitude of activation was greater in the control compared to cKO mice (Figure 2D, left), although response latency did not differ between the two groups (Figure 2E, left). VTA optogenetic stimulation inhibited some VStr neurons; the cKO mice had significantly fewer neurons inhibited and greater inhibition latency than the control mice, whereas the two groups did not differ in unit inhibition magnitude (Figures 2C and 2D, right).



**Figure 2. cKO of VGLUT2 from DA Neurons Reduces VStr Phasic Response**

(A) Summary of the z-scored perievent histogram of all the recorded VStr neurons upon VTA optogenetic stimulation in Control ( $n = 93$ ; left panel) and cKO mice ( $n = 112$ ; right). Perievent histograms (bin size, 5 ms) were smoothed with a Gaussian filter (filter width, 3 bins). Color bars represent z-scored firing frequency. (B) Cumulative percentages of the activated (red) and inhibited (blue) VStr neurons with response latencies of 200 ms or less, recorded from control (filled circles) and cKO mice (open circles).

(C) Percentages of the same four groups of neurons (as shown in B) that responded with latencies of 50 ms or less. A chi-square test was used for statistics. Ctl, control.

(D and E) Response amplitude (D; mean  $\pm$  SEM) and response latency (E; mean  $\pm$  SEM) of the same four groups of neurons that responded with latencies of 50 ms or less. An unpaired two-tailed t test was used for statistics.

### cKO of VGLUT2 Reduces Glutamate Release In Vivo and Eliminates Glutamate Co-transmission In Vitro

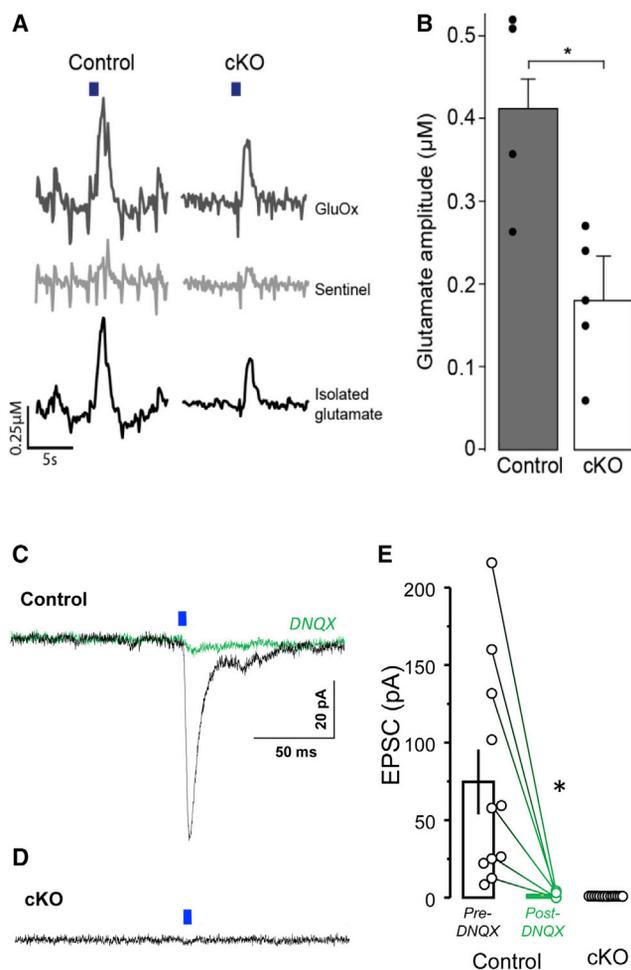
To verify attenuated glutamate release in cKO mice, we performed amperometric measurements of VStr glutamate evoked by terminal stimulation of VTA-VStr DA neurons. We observed reduced glutamate release in cKO mice (Figures 3A and 3B; Tables S1 and S2), although optogenetic stimulation still evoked some glutamate release in cKO mice.

To determine whether the residual responses in the VStr of the cKO mice (Figure 2) may be mediated by incomplete knockout of VGLUT2 in DA neurons, we performed whole-cell recordings from VStr medium spiny neurons using acute brain slice preparations. Optogenetic stimulation of ChR2-expressing DA terminals evoked excitatory post-synaptic currents (EPSCs) in all recorded neurons of control mice, and the evoked EPSCs, measured at  $V_h$  of  $-70$  mV, were blocked by the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) (Figures 3C and 3E). By contrast, we did not detect light-evoked EPSCs in any VStr recorded neurons of cKO mice (Figures 3D and 3E). These results suggest that, as shown for other cKO lines (Stuber et al., 2010; Tritsch et al., 2012), the co-transmission of glutamate is eliminated after the cKO of VGLUT2. Therefore, the

fast-excitatory responses in striatal neurons of cKO mice in vivo are mediated by mechanisms distinct from glutamate co-release, and the evoked glutamate release detected over 1–2 s is likely explained by network effects induced by release of DA and co-transmitters such as  $\gamma$ -aminobutyric acid (GABA) and cholecystokinin (CCK) from DA neurons.

### Glutamate Co-transmission Has Little Effect on the Acquisition of Conditioned Behavior

We used naive mice to examine whether glutamate co-transmission is important in acquisition of behavior reinforced by phasic activation of VTA DA neurons in an ICSS procedure. Mice significantly increased lever presses reinforced by VTA optogenetic stimulation (a 50-Hz eight-pulse train; 8-mW intensity and 1-ms pulse width) over the first five sessions (session effect:  $F_{(4,84)} = 11.60$ ,  $p < 0.00001$  with a  $2_{\text{group}} \times 5_{\text{session}}$  mixed ANOVA), whereas cKO and control mice increased lever presses in a similar manner (group effect:  $F_{(1,21)} = 0.58$ ,  $p = 0.58$ ; group  $\times$  session interaction:  $F_{(4,84)} = 0.096$ ,  $p = 0.98$ ) (Figures 4A–4C). To recruit more neurons for greater response rates, we let mice lever-press for optogenetic stimulation of greater intensity (32 mW and 3-ms pulse width) over sessions 6–10. Although lever presses further increased (session effect:  $F_{(4,84)} = 35.65$ ,  $p < 0.00001$



**Figure 3. cKO of VGLUT2 Reduces Extracellular Glutamate Release In Vivo and Eliminates Glutamate Co-transmission In Vitro**

(A) Optogenetic stimulation (20 pulses at 20 Hz; blue bar) of VTA DA neuron terminals in the VStr evoked glutamate release in control (left) and cKO (right). Raw signals obtained from the GluOx-coated (top) and sentinel channel (middle) were subtracted from one another to obtain an isolated glutamate signal (bottom).

(B) The cKO mice ( $n = 5$ ) had significantly lower glutamate amplitude than the control mice ( $n = 4$ ) ( $t = 3.384$ ,  $df = 7$ ,  $p = 0.0117$ ; unpaired two-tailed  $t$  test).

(C and D) Optogenetic light pulse (5 ms) delivered at DA neuron terminals evoked EPSCs from VStr neurons ( $V_h = -70$  mV) of control (C), but not cKO (D), mice. The EPSCs present in controls were completely blocked by the AMPA receptor antagonist DNQX (C; green trace).

(E) Summary of EPSC peak amplitudes recorded from control ( $n = 11$  neurons;  $n = 2$  mice) and cKO ( $n = 14$  neurons;  $n = 2$  mice) mice.

with a  $2_{\text{group}} \times 5_{\text{session}}$  mixed ANOVA) without a main group effect ( $F_{(1,21)} = 0.025$ ,  $p = 0.88$ ), a significant group  $\times$  session interaction was found ( $F_{(4,84)} = 5.03$ ,  $p = 0.006$ ). In addition, we examined how the mice change response rates as a function of optogenetic stimulation value. We used three different parameters of optogenetic stimulation (three, five, and eight pulses delivered at 14, 29, and 50 Hz, respectively). cKO and control mice displayed similar patterns of responding as a function of optogenetic stimulation value, with higher response rates for

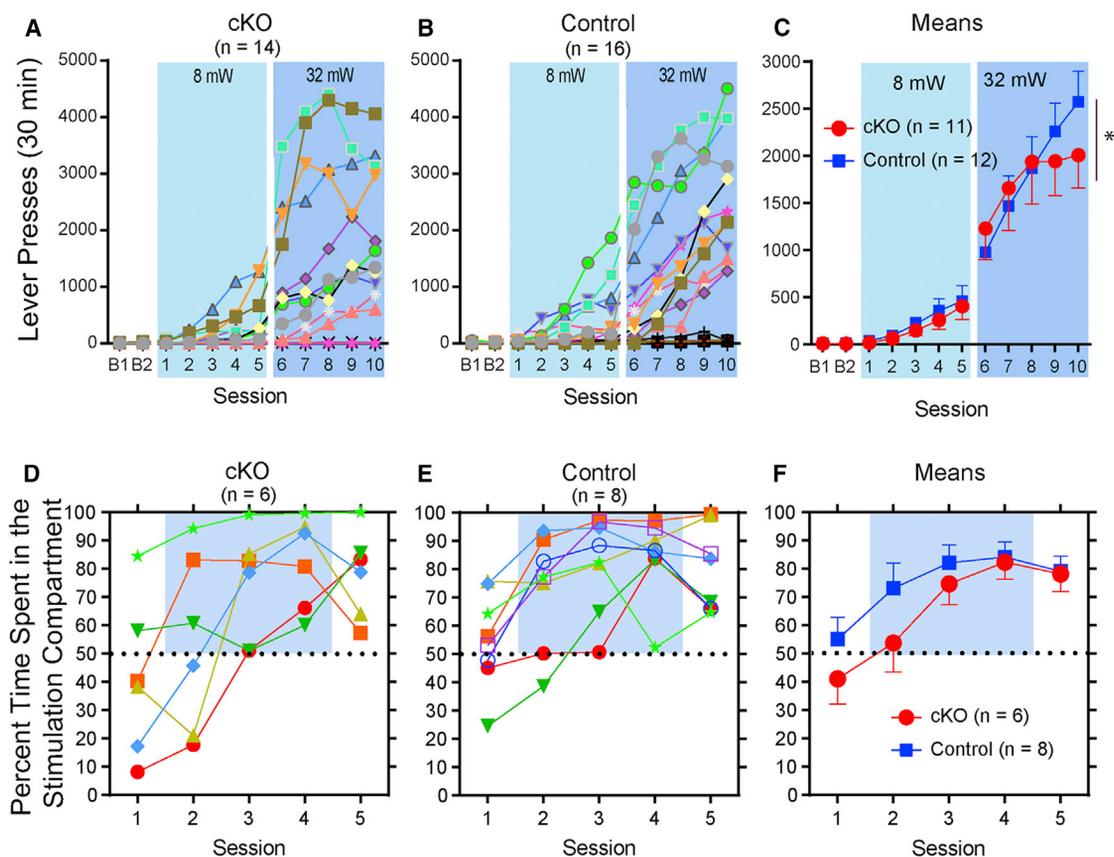
stimulations with greater pulse number-frequency (Figure S4). These results suggest that the loss of glutamate co-transmission from DA neurons does not affect sensitivity to reward value produced by DA neuron activation.

We also examined whether cKO mice acquire real-time place preference induced by optogenetic stimulation of VStr terminals of VTA DA neurons. Such stimulation (continuous 20 Hz) did not produce a significant difference in real-time place preference between the cKO and littermate control mice (Figures 4D–4F) (session effect:  $F_{(1,12)} = 333.41$ ,  $p < 0.00001$ , with a  $2_{\text{group}} \times 5_{\text{session}}$  mixed ANOVA; group effect:  $F_{(1,12)} = 1.27$ ,  $p = 0.28$ ; group  $\times$  session interaction:  $F_{(1,12)} = 0.99$ ,  $p = 0.42$ ). These results suggest that the loss of glutamate co-transmission does not affect the acquisition of place preference reinforced by the stimulation of DA terminals in the VStr.

## DISCUSSION

We discuss three notable methodological issues here. First, the use of DAT-Cre mice, especially the controls ( $Slc17a6^{+/flox}; Slc6a3^{+/IRES-Cre}$ ), served as a complement for the experiment where TH-Cre mice were used, and vice versa. This is because the TH-Cre line expresses Cre in some non-DA neurons (Lammel et al., 2015; Lindeberg et al., 2004; Nordenankar et al., 2015; Pupe and Wallén-Mackenzie, 2015), and some TH-expressing VTA neurons, particularly those located in the medial VTA, do not express DAT (Lammel et al., 2015; Viereckel et al., 2016). Second, a 50-Hz train employed in the present study was adequate for examining fast activation of striatal neurons despite the reports that 50-Hz optogenetic stimulation does not trigger reliable depolarization at each pulse in vitro (Tsai et al., 2009; Witten et al., 2011). This is because (1) many DA neurons can spontaneously fire 50 Hz or more (Wang and Tsien, 2011); (2) 50-Hz optogenetic stimulation can trigger a spike for each pulse in vivo and supports vigorous ICSS (Ilango et al., 2014a); (3) 40-Hz optogenetic stimulation triggers greater DA release than 20- or 30-Hz stimulation in vivo (Bass et al., 2010). Finally, although the VTA AAV injections resulted in the expression of ChR2 in some medial SNc DA neurons, optogenetic stimulation most likely activated selectively VTA DA neurons. This is because optogenetic stimulation only excites neurons just below the tip. Even if it recruited nigral DA neurons, fast excitatory activation must have been mediated by VTA DA neurons because nigral DA cells do not express detectable levels of VGLUT2 (Hnasko et al., 2010) or project densely to the VStr (Ikemoto, 2007).

The present study observed that stimulation of VTA DA neurons activates many VStr neurons within 50 ms in freely moving adult mice (Figures 1D and 2B), and this observation is most likely explained by glutamate co-transmission of DA neurons. First, these excitatory responses were not mediated by DA, because DA receptors are G-protein coupled, with relatively slow activation kinetics ( $>50$  ms) (Dong and White, 2003; Marcott et al., 2014). Consistently, these evoked fast responses were still present after the administration of DA D1 or D2 receptor antagonist (Figure S3). Second, optogenetic stimulation of VTA DA neurons excited fewer VStr neurons and resulted in less glutamate release (with VTA-VStr terminal stimulation) in cKO mice than control mice.



**Figure 4. cKO of VGLUT2 from DA Neurons Does Not Affect the Acquisition of ICSS or Place Preference**

(A and B) Active lever pressing was reinforced with VTA optogenetic stimulation (50 Hz, eight pulses with 1-ms pulse width; 8 mW) in sessions 1–5 and then with optogenetic stimulation of increased intensity (3-ms pulse width; 32 mW) in sessions 6–10. In session 10, 11 out of 14 cKO (A) and 12 out of 16 control mice (B) responded 500 times or more.

(C) Mean responses with SEM are shown with the data of responders with 500 responses or more. The cutoff of 500 responses is to focus on differences in responders of the two groups and is justified by the fact that similar ratios of mice were excluded from the analyses between the two groups. \* $p < 0.05$ , a significant group  $\times$  session interaction.

(D and E) Although no optogenetic stimulation was delivered in sessions 1 or 5, cKO (D) and control (E) mice received VStr optogenetic stimulation in one of two compartments during sessions 2–4. Depicted are percent time spent in the stimulation compartment of each mouse.

(F) Percentage of time (mean  $\pm$  SEM) spent in the stimulation compartment.

Although the residual fast-excitatory responses and glutamate release in response to light-evoked DA neuron stimulation raised a concern as to whether the cKO of VGLUT2 was complete, whole-cell recordings from VStr medium-sized spiny neurons show clearly the complete removal of glutamate co-transmission in cKO mice. Therefore, any remaining fast excitation must be explained by other factors that may include (1) background/signal noise, (2) alternate excitatory signal/transmitter, and (3) polysynaptic mechanism. Similarly, the evoked glutamate release, which took place on the scale of seconds, must be due to secondary, tertiary, and quaternary consequences mediated by DA and other co-transmitters such as GABA or neuropeptides. This is consistent with our amperometric measurements that did not detect synaptic glutamate release but extra-synaptic levels of glutamate over 1–2 s.

We unexpectedly observed that optogenetic stimulation of DA neurons inhibited a smaller percentage of VStr neurons in the

cKO mice than the control mice, and the latency of inhibitory responses was greater in the cKO mice than the control mice. These observations can be understood with consideration of previous findings. First, DA neurons have been shown to co-transmit GABA (Tritsch et al., 2012, 2014). In addition, 99% of striatal neurons are GABAergic, and they receive inhibitory inputs from not only interneurons but also medium-sized spiny neurons, which send extensive collaterals to each other and engage in mutual inhibitory interactions (Bishop et al., 1982; Taverna et al., 2004). Accordingly, the elimination of fast excitation in GABAergic neurons in cKO mice could result in the elimination of secondary inhibitory responses among striatal neurons.

The present study showed that the cKO mice acquired conditioned responding reinforced by VTA DA neuron activation at similar rates as control mice. Similarly, previous studies found no clear difference between cKO and control mice in acquiring

motor performance with rotarod or operant responding reinforced by sucrose pellets, or performing a radial maze baited with food (Birgner et al., 2010; Hnasko et al., 2010). Although these tasks arguably depend, at least in part, on DA neuron activity (Alsiö et al., 2011; Birgner et al., 2010; Hnasko et al., 2010), the present study demonstrated dispensability of glutamate co-transmission in trial-and-error learning with responding reinforced by DA neuron activation.

A subtle difference between cKO and control mice emerged after many sessions of ICSS. Although both groups increased ICSS rates for each subsequent session, the rate of increase was lessened for cKO groups during the last several sessions when compared to controls. The difference may be explained by the role of glutamate in vesicular loading of DA (Hnasko et al., 2010; Hnasko and Edwards, 2012). Indeed, VGLUT2 cKO from DA neurons not only eliminates glutamate release but also reduces DA release (Alsiö et al., 2011; Fortin et al., 2012; Hnasko et al., 2010). Because DA transmission is important in energizing behavior (Hamid et al., 2016; Ilango et al., 2014a), the deficit in DA vesicular loading may have prevented the cKO mice from performing at high response rates. Consistently, previous studies found that cKO mice did not increase locomotor activity as much as control mice after the administration of a low dose of amphetamine or cocaine (Birgner et al., 2010; Hnasko et al., 2010). Moreover, cKO mice self-administer more cocaine than control mice (Alsiö et al., 2011). The latter effect is explained by the fact that cocaine increases extracellular DA concentration by blocking DA uptake, and DA concentration regulates cocaine self-administration (Norman and Tsibulsky, 2006; Wise et al., 1995). Therefore, reduced DA concentrations in the cKO may cause mice to administer cocaine with shorter intervals (Ikemoto et al., 2015). Interestingly, cocaine is found to blunt glutamate co-transmission from DA neurons via enhanced activation of D2 autoreceptors (Adrover et al., 2014), corroborating the notion that glutamate co-transmission plays little role in vigor of behavior.

In sum, we provide direct evidence that glutamate co-transmission from DA terminals can trigger fast excitatory responses of VStr neurons in vivo. However, despite the asymptotic response difference between cKO and control mice in ICSS, acquisition rates of ICSS and real-time place preference did not differ between the two groups. Therefore, glutamate co-transmission appears dispensable for the acquisition of conditioned responding reinforced by DA neuron activation.

## EXPERIMENTAL PROCEDURES

Detailed protocols are described in the [Supplemental Experimental Procedures](#).

### Animals

TH-IRES-Cre mice, cKO mice (Slc17a6<sup>flox/flox</sup>; Slc6a3<sup>+IRESCre</sup>), and control mice (Slc17a6<sup>+flox</sup>; Slc6a3<sup>+IRESCre</sup>) were used.

### ICSS

Two levers, an active and an inactive, were available in the self-stimulation chamber. During the acquisition phase, press of the active lever triggered eight pulses of 50-Hz optogenetic stimulation in the VTA (no timeout except for the duration of optogenetic stimulation), whereas press of the inactive lever had no consequence. A lower laser intensity (8 mW and 1-ms pulse width) was used

between acquisition sessions 1 and 5 (all three mouse lines), and a higher laser intensity (32 mW and 3-ms pulse width) was used between acquisition sessions 6 and 10 (only DAT and cKO mice).

### In Vivo Electrophysiology

We used a bundle of eight tetrodes (32 channels) coupled to a moveable (screw-driven) microdrive assembly for in vivo VStr recording in freely behaving mice. Neural signals were recorded using a Neuralynx Digital Lynx acquisition system. Spikes were digitized at 32 kHz and filtered at 600–6,000 Hz, using one recording electrode that lacked obvious spike signals as the reference.

### In Vitro Electrophysiology

Acute brain slices were prepared, and whole-cell recordings were made from visually identified VStr neurons held in voltage clamp at  $-70$  mV to record AMPA receptor (AMPA) EPSCs as previously described (Yoo et al., 2016). For whole-cell voltage-clamp recordings, single-pulse (5-ms) optogenetic stimuli were applied every 55 s and 10 photo-evoked currents were averaged per neuron per condition.

### In Vivo Amperometry

The microelectrode consisted of a ceramic paddle with four platinum recording sites. One pair of recording sites was coated with a mixture of GluOx (2%, 0.5 unit/1  $\mu$ L), BSA (1%), and glutaraldehyde (0.125%). The other pair was coated only with BSA (1%) and glutaraldehyde (0.125%) to serve as control (sentinel/background) channels. Glutamate recordings were performed in the VStr under continuous isoflurane anesthesia with 5 trains of photostimulation (1 s at 20 Hz; 4–8 mW) separated by 30 s at +0.7V to control for possible light-induced artifacts, the photostimulation protocol was repeated at +0.25V.

### Place Preference

We used home-built two-chamber acrylic apparatuses. Time spent in each chamber was assessed, and mice were assigned active (i.e., optogenetic stimulation-paired) sides in an unbiased manner. Upon head entry to the pre-defined active side, software triggered transistor-to-transistor logic (TTL) pulses to activate the laser (20 Hz, 10-ms pulse width,  $\sim 10$  mW at fiber tip), which discontinued upon head exit.

### Data Analysis

#### Tetrode Recording

We used the Plexon OfflineSorter for separation of recorded spike waveforms. Sorted spikes were processed and analyzed in NeuroExplorer (Nex Technologies) and MATLAB (Mathworks). For z-score analysis, neural activity 1 s before the optogenetic stimulation was used as the baseline. Response latency was defined as the latency of the first bin from at least five consecutive bins that exceeded the z score of 2 (or  $-2$  for inhibited neurons).

### Histology

Mice were deeply anesthetized and intracardially perfused with PBS followed by 4% paraformaldehyde (PFA). Brains were sliced on a cryostat (50- $\mu$ m coronal sections) or a vibratome (60- $\mu$ m coronal sections). Sections were mounted with the Mowiol mounting medium mixed with DAPI.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2017.02.062>.

## AUTHOR CONTRIBUTIONS

D.V.W. and S.I. designed self-stimulation and electrophysiological experiments, analyzed the data, and drafted the manuscript; D.V.W., C.J.B., A.T., and A.J.K. performed the experiments. T.S.H., J.H.Y., and M.H.G. designed and performed the place preference experiment. T.S.H. and V.Z. designed and performed the slice electrophysiology experiment. T.V., Å.K.G., and

Å.W-M. designed amperometry experiments and analyzed data; T.V., E.A., and Å.K.G. performed the experiments.

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