

## An Integrative Perspective on the Role of Dopamine in Schizophrenia

### *Supplemental Information*

#### **PREDICTIONS AND FURTHER TESTS OF THE HYPOTHESES THAT SCHIZOPHRENIA INVOLVES INCREASED SPONTANEOUS AND DECREASED ADAPTIVE TRANSIENTS**

The main strength of the hypotheses, advanced in this article, that schizophrenia involves (a) increased spontaneous transients, which relate to positive symptoms, and (b) decreased adaptive transients, which relate to negative symptoms, is the extensive set of findings that they explain (see Figs. 4–5 in the article). Directly and more definitively testing these hypotheses would ideally involve measuring dopamine transients in the striatum of patients with schizophrenia (vs. healthy controls), both at rest, to measure spontaneous transients, and during reward-related learning, to measure adaptive transients. Unfortunately, limitations in current imaging technology mean that noninvasively measuring dopamine transients *in vivo* in the striatum in humans is not possible: molecular-imaging techniques, such as PET and SPECT, are too slow to capture these transients, and faster techniques, such as fMRI, do not measure neurochemistry. Nonetheless, these hypotheses lead to several, less direct, predictions that can be tested experimentally (some of which are listed below). Given that such tests are indirect, none of them individually, nor, indeed, all of them together, will be sufficient to definitively prove or disprove these hypotheses. Nonetheless, they would add to the existing body of circumstantial evidence, on a par with the evidence already reviewed in this article, thereby helping to strengthen further or instead weaken confidence in these hypotheses.

The following provides a list of possible indirect tests of these hypotheses:

1. *Testing, in animals, if ketamine and other psychotomimetic drugs increase spontaneous transients.* If psychosis is caused by increased spontaneous transients, then psychotomimetic drugs from classes other than psychostimulants—e.g., ketamine and

other non-competitive NMDA antagonists—may also increase spontaneous transients. Ketamine and phencyclidine increase spontaneous firing and spontaneous bursts of dopamine neurons (1,2), so it seems likely that they may indeed increase spontaneous dopamine transients in the striatum. However, this hypothesis should be tested explicitly.

2. *Testing, in animals, if ketamine and other drugs that cause negative symptoms blunt adaptive transients.* Ketamine and other non-competitive NMDA antagonists have the interesting characteristic of producing not only positive but also negative symptoms (3,4). If negative symptoms are caused, at least in part, by decreased adaptive transients, then ketamine and other drugs that produce negative symptoms may blunt adaptive transients.
3. *Testing, using resting-state fMRI in patients, if psychosis is associated with increased functional connectivity between dopamine regions in the midbrain (substantia nigra pars compacta, SNc, and ventral tegmental area, VTA) and the striatum.* If psychosis is caused by increased spontaneous transients, then the functional connectivity between the SNc/VTA and the striatum might be increased at rest in psychosis. This prediction could be tested in multiple ways, including by comparing psychotic schizophrenia patients with healthy controls (and possibly with non-psychotic stabilized patients); following subjects at ultra-high-risk for psychosis longitudinally and seeing if the baseline level of SNc/VTA functional connectivity predicts who will convert to psychosis, or if that connectivity increases as patients convert to psychosis; and with several other experimental designs.
4. *Characterizing, in animals, the effects of antipsychotics on spontaneous dopamine transients.* If psychosis is caused by increased spontaneous dopamine transients, decreasing those transients—or their postsynaptic effects—might be crucial for the effectiveness of antipsychotics against positive symptoms. Antipsychotics blunt firing of dopamine neurons through depolarization block (5). In normal animals, this effect takes approximately three weeks to develop (5), which seems inconsistent with the immediate effects of antipsychotics (6,7). However, in an animal model of schizophrenia,

depolarization block occurs quickly—in fact, even with acute administration (8). Does the depolarization block translate into reduced spontaneous dopamine transients in the striatum? Do all antipsychotics—typical and atypical—decrease those spontaneous transients? If so, do the doses of different antipsychotics required to bring the level of spontaneous transients in animal models of schizophrenia into a normal range correlate with the doses required for clinical effectiveness against psychosis?

5. *Testing, in animals, the effects on spontaneous and adaptive dopamine transients of genetic mutations associated with schizophrenia in humans.* An example of the potential of this approach comes from a study in which a mutation possibly associated with schizophrenia, affecting the calcium-activated small conductance potassium channel SK3, was expressed selectively in dopamine neurons in mice (9). As a result, mice exhibited increases in (a) spontaneous bursts of dopamine neurons during free movement, (b) increased dopamine release, and (c) increased sensitivity to MK-801, a drug commonly used to induce schizophrenia-like symptoms in animal models (which, like ketamine, is a non-competitive NMDA antagonist). Although the relevance of this particular mutation and gene to schizophrenia is still under debate, the substantial recent interest in the role of *de novo* and rare disruptive mutations in schizophrenia (10–12), together with the likely future developments in this area, should make this approach increasingly feasible and important.

This list is not intended to be exhaustive in any way. Instead, it aims to provide an illustrative sample of possible tests, hoping to invigorate a research program that tests these hypotheses.

As mentioned above, more direct tests of these hypotheses would require measuring dopamine transients in the striatum of patients with schizophrenia (vs. healthy controls). Such direct measurements are now, in fact, possible, using fast-scan cyclic voltammetry (FSCV), but only intra-operatively (13). Should the opportunity arise to ethically and safely apply these techniques to patients with schizophrenia undergoing brain surgery for some other indication, or

should deep-brain stimulation, possibly involving the striatum, become useful for the treatment of schizophrenia (14), these FSCV techniques could potentially be used to directly test our hypotheses (by comparing patients with schizophrenia with other patients).

## **SPONTANEOUS AND ADAPTIVE BURSTING IN DOPAMINE NEURONS AND SCHIZOPHRENIA**

### **Spontaneous Bursts in Dopamine Neurons**

Dopamine neurons exhibit spontaneous burst-firing in the absence of identified external stimuli in freely moving (15) and even anesthetized animals (16). The electrophysiological properties of these bursts are indistinguishable from those of bursts elicited by relevant stimuli in behaving animals (17). These bursts generally depend on stimulation of NMDA receptors in dopamine neurons (17) but are also elicited by intracellular calcium injection (16) or by activating L-type voltage-gated calcium channels (LTCCs)—specifically,  $Ca_v1.2$  and  $Ca_v1.3$  channels—in dopamine neurons (18).

### **Mechanisms Underlying Increased Spontaneous Transients and Decreased Adaptive Transients in Schizophrenia**

#### *An Illustrative Example: Genetic Alterations in $Ca_v1.2$ Channels*

The mechanisms underlying increased spontaneous and decreased adaptive transients in schizophrenia are unknown. Here, we will use disturbances in  $Ca_v1.2$  channels as an example of an alteration that is suggested by genetic findings and that *could* cause both increased spontaneous and decreased adaptive transients. This is just an illustrative example, though: the goal is to show that the coexistence of increased spontaneous and decreased adaptive transients is realistic mechanistically—an important idea even if the mechanisms causing these disturbances in schizophrenia turn out to be different. In fact, given the highly polygenic nature of schizophrenia (10,19), we suspect that many mechanisms are involved.

As mentioned above, activation of  $Ca_v1.2$  channels in dopamine neurons increases spontaneous bursts (18). Interestingly, *CACNA1C*, the gene that codes a key subunit of  $Ca_v1.2$  channels, has repeatedly been associated with schizophrenia (10,19,20). The studies examining the effects of the risk allele on *CACNA1C* gene expression have yielded contradictory findings (21), but a study that used induced human neurons found that the homozygous risk allele not only increased *CACNA1C* mRNA but also increased calcium current through  $Ca_v1.2$  channels (22). Such increased calcium current may increase spontaneous bursting, just as pharmacological activation of  $Ca_v1.2$  channels (18) or direct calcium injection into dopamine neurons (16) do. Consistent with these ideas, there is some, albeit limited, evidence for a beneficial effect of LTCC antagonists in schizophrenia (23). Furthermore, repeated amphetamine administration, which increases the risk for psychosis, upregulates  $Ca_v1.2$  channels in dopamine neurons (24). Notably, pharmacological activation of LTCCs also increases dopamine efflux, synthesis, and turnover in the striatum (25,26), consistent with equivalent PET findings in schizophrenia.

Healthy individuals with the *CACNA1C* risk allele exhibit reduced learning from rewards (27), like patients with schizophrenia do. Thus, a risk allele for schizophrenia that may increase spontaneous bursting may simultaneously blunt reward learning. Whether this risk allele blunts adaptive bursts is unknown. However, rodents with a gain-of-function mutation in *CACNA1C* show activity-dependent dendritic retraction, rather than the activity-dependent dendritic growth shown by wildtype animals (28): an abnormality in synaptic plasticity that would be expected to impair learning.

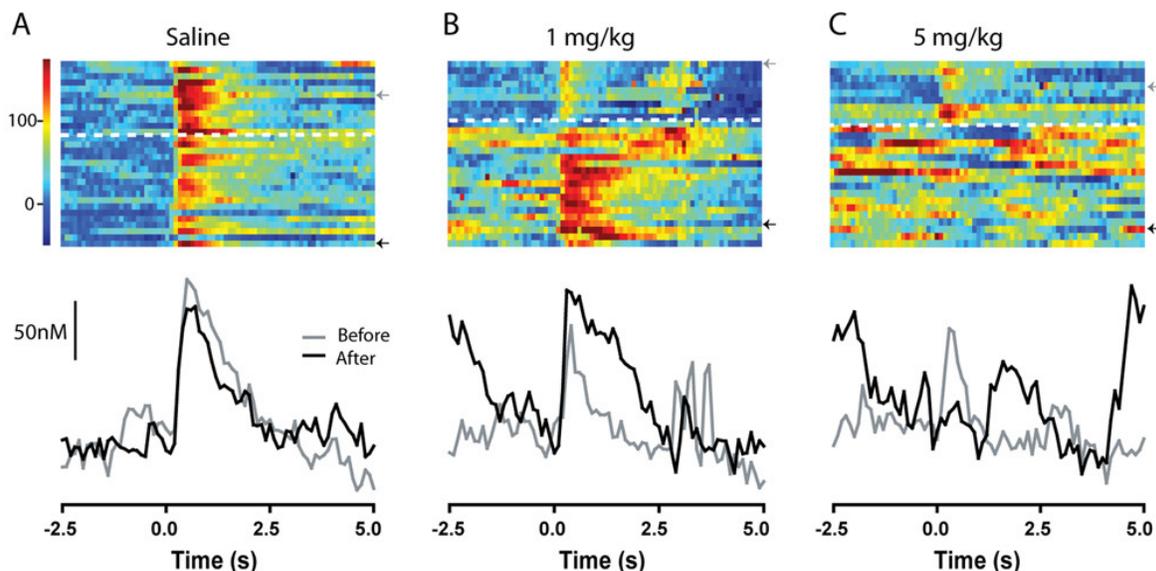
The *CACNA1C* risk allele may therefore provide a tantalizingly coherent mechanistic account for the disturbances that we propose underlie schizophrenia. Of course such mechanistic account, even if correct, is necessarily far from complete: the *CACNA1C* risk allele has a small effect size, and it is present in about one third of the general population (21), so it likely plays a modest role in schizophrenia. Many other genes commonly implicated in

schizophrenia, however, also involve voltage-gated calcium channels or the NMDA receptor (10,19), both of which modulate burst firing in dopamine neurons.

*Increased Spontaneous Transients as the Direct Cause of Decreased Adaptive Transients*

Increased spontaneous and decreased adaptive transients might have a common cause, genetic or otherwise. Alternatively, increased spontaneous transients could themselves cause decreased adaptive transients—e.g., by stimulating inhibitory D2 autoreceptors. Another possibility is that increased spontaneous transients might not reduce adaptive transients but rather “drown out” adaptive transients (29) by decreasing their signal-to-noise ratio. This hypothesis would explain the reduced BOLD responses for rewards and reward-predicting stimuli in schizophrenia (29) because BOLD responses are always measured by comparison against a baseline. Whether it would also explain findings such as blunted Go learning in schizophrenia depends on whether Go learning is determined primarily by the difference between adaptive transients and dopamine levels prior to those transients or instead by the absolute level of dopamine during adaptive transients. To the best of our knowledge, this question remains unresolved. Repeated spontaneous transients could also, over time, conceivably lead to postsynaptic adaptations that would reduce the postsynaptic effect of transients (spontaneous or adaptive).

Contrary to the idea that adaptive transients themselves may be preserved, some evidence suggests that, at least in the case of high doses of amphetamine, adaptive transients may truly be reduced, rather than just being more difficult to detect amongst increased spontaneous transients (30) (Fig. S1). Nonetheless, additional research will be necessary to allow more definitive conclusions on these issues.



**Figure S1.** Representative recordings of the effects of amphetamine on adaptive dopamine transients elicited by a reward-predicting cue, measured using fast-scan cyclic voltammetry. The cue is presented at time 0. In the top panels, each row corresponds to a trial, the colors represent dopamine levels (with hotter colors representing more dopamine), and the horizontal dashed white line represents the time of drug injection. In the bottom panels, gray lines represent pre-drug dopamine levels, and black lines represent dopamine levels after drug injection. **(A)** Both before and after saline injection, the reward-predicting cue elicits strong adaptive dopamine transients. **(B)** A moderate dose of amphetamine increases both the peak and duration of adaptive transients (compare the gray and black lines). **(C)** A high dose of amphetamine increases spontaneous transients (i.e., transients that are not appropriately time-locked to the cue: note the hot colors at multiple points in the trial), and it decreases adaptive transients (compare the gray and black lines in the first second after cue presentation: the adaptive transient was substantially greater before than after drug injection). Adapted, with permission, from Ref. (30).

#### *Increased Spontaneous Transients and Decreased Adaptive Transients due to Inappropriate Driving of Dopamine Responses by Stimuli*

Increased spontaneous and decreased adaptive transients could also result from inappropriate driving of dopamine responses by stimuli, as a consequence of higher-level disturbances, as discussed in section “Relation to Other Deficits and Neural Systems” of the article.

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