Transient Calcium and Dopamine Increase PKA Activity and DARPP-32 Phosphorylation

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Reinforcement learning theorizes that strengthening of synaptic connections in medium spiny neurons of the striatum occurs when glutamatergic input (from cortex) and dopaminergic input (from substantia nigra) are received simultaneously. Subsequent to learning, medium spiny neurons with strengthened synapses are more likely to fire in response to cortical input alone. This synaptic plasticity is produced by phosphorylation of AMPA receptors, caused by phosphorylation of various signalling molecules. A key signalling molecule is the phosphoprotein DARPP-32, highly expressed in striatal medium spiny neurons. DARPP-32 is regulated by several neurotransmitters through a complex network of intracellular signalling pathways involving cAMP (increased through dopamine stimulation) and calcium (increased through glutamate stimulation). Since DARPP-32 controls several kinases and phosphatases involved in striatal synaptic plasticity, understanding the interactions between cAMP and calcium, in particular the effect of transient stimuli on DARPP-32 phosphorylation, has major implications for understanding reinforcement learning. We developed a computer model of the biochemical reaction pathways involved in the phosphorylation of DARPP-32 on Thr34 and Thr75. Ordinary differential equations describing the biochemical reactions were implemented in a single compartment model using the software XPPAUT. Reaction rate constants were obtained from the biochemical literature. The first set of simulations using sustained elevations of dopamine and calcium produced phosphorylation levels of DARPP-32 similar to that measured experimentally, thereby validating the model. The second set of simulations, using the validated model, showed that transient dopamine elevations increased the phosphorylation of Thr34 as expected, but transient calcium elevations also increased the phosphorylation of Thr34, contrary to what is believed. When transient calcium and dopamine stimuli were paired, PKA activation and Thr34 phosphorylation increased compared with dopamine alone. This result, which is robust to variation in model parameters, supports reinforcement learning theories in which activity-dependent long-term synaptic plasticity requires paired glutamate and dopamine inputs.

Introduction

The basal ganglia play an important role in reinforcement learning, in which an animal learns that performing a particular action in response to a neutral (e.g., visual) stimulus will be rewarded [1,2]. In vivo recordings during learning reveal that the reward elicits an increase in dopamine neuron firing [3], and an increase in dopamine release in the striatum [4,5], whereas the visual stimulus and the motor action produce cortical activity, which is transmitted to the striatum as an increase in glutamate release. Thus, during reinforcement learning, the medium spiny neurons of the striatum receive paired glutamate and dopamine input. Numerous studies show that glutamate input from cortex combined with dopamine produces a persistent increase in the size of the glutamatergic EPSC of medium spiny neurons, which is known as long-term potentiation (LTP). These observations support the hypothesis that synaptic plasticity of medium spiny neurons underlying reinforcement learning: the cortico–striatal synapses that are active simultaneously with dopamine input are potentiated during reinforcement learning [6,7]. Equally important to theoretical models of reinforcement learning is that synaptic potentiation does not occur in response to dopamine or glutamate signals alone [8]. Nonetheless, the subcellular mechanisms within medium spiny neurons underlying the requirement for paired stimuli are not completely understood.

Synaptic plasticity is controlled by the state of phosphorylation of various components in the intracellular signalling network [9,10]. Phosphorylation of Ser 845 on the GluR1 subunit by cAMP-dependent kinase (PKA) increases insertion of AMPA receptors into the membrane, whereas dephosphorylation by protein phosphatase 1 (PP1) has the opposite effect [11,12]. In the medium spiny neurons of the striatum, the balance between PKA and PP1 is heavily regulated by the

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Synopsis

Reinforcement learning, based on the association of a stimulus-triggered movement with a reward, involves changes in connection strength between neurons. Memory storage occurs in the striatum, the input stage of the basal ganglia, when a stimulus or movement signal originating from the cortex and a reward signal originating from the midbrain reach the target striatal cells together. Repetitive pairing of these two signals strengthens the connection between cortical and striatal cells. The strengthening of the connections is caused by activation of biochemical signalling pathways inside the striatal cells. These intracellular signalling pathways are explored in a quantitative computational model describing the biochemical pathways important for reinforcement learning. Lindskog et al.’s study shows that when brief reward and stimuli signals are paired, a stronger response in the intracellular signalling occurs compared with the situation when each signal is given alone. This result illustrates mechanisms whereby paired stimuli, but not unpaired stimuli, can cause learning. Furthermore, the model predicts that the biochemical responses are different after brief stimulation as compared with prolonged stimulation. This result highlights the difficulties in predicting the nonlinear interactions within signalling cascades based on prolonged stimulations, which are often used in biochemical experiments.

Phosphoprotein DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa) [13]. The activity of DARPP-32 is regulated mainly by two phosphorylation sites: threonine (Thr) 34 and Thr 75 (rat sequence). When phosphorylated at Thr34, DARPP-32 is a potent inhibitor of PP1 [14], whereas when phosphorylated at Thr75 DARPP-32 inhibits protein kinase A (PKA) [15]. The state of phosphorylation of DARPP-32 has been shown to be regulated by dopamine and several other neurotransmitters, including glutamate [16,17], adenosine [18,19], and opioids [20]. Thr34 is phosphorylated by PKA and dephosphorylated by protein phosphatase 2B (PP2B or calcineurin) [16,21] whereas Thr75 is phosphorylated by the cyclin-dependent kinase 5 (cdk5) and dephosphorylated mainly by PP2A [15,17]. A vast amount of data regarding the regulation of phosphorylation of DARPP-32, as well as its effect on other intracellular proteins, has been generated in the last few years (for review see [13]). Nonetheless, it is not evident how co-activation of multiple neurotransmitters modulates the signalling network.

To gain a better understanding of the mechanisms regulating DARPP-32 phosphorylation and its effect on kinase and phosphatase activity and subsequent plasticity, we developed a computer model of this signalling network, based on the available experimental data. The model allows us to investigate the effect of transient glutamate and dopamine stimuli on levels of signalling molecules and the phosphorylation of DARPP-32; and to identify critical reactions within the network. We find that transient stimulation produces different enzyme activation compared with the prolonged treatments typically used in biochemical experiments. For example, short pulses of calcium influx increase DARPP-32 phosphorylation at Thr34 compared with the decrease seen with prolonged stimulation. Another finding is that the feedback loop PKA–PP2A–phosphoThr75 does not exclusively reinforce the PKA pathway, as previously hypothesized, but at times acts as a sink for catalytic PKA, dampening the stimulatory effect of dopamine.

Results

Model Verification: Response to Steady-State Inputs

The model, illustrated in Figure 1, consists of ordinary differential equations describing mass action kinetics of biochemical reactions known to occur in striatal medium spiny neurons. Tables 1–4 list these reactions, as well as their rate constants and the quantities of molecules used in the model. Equations are derived assuming all reactions are in a single compartment (i.e., a spine).

Several simulations were designed to replicate biochemical experiments in order to compare the model results with “steady-state” experiments, in which the phosphorylation levels were measured after a few minutes. Some simulations were performed for the final adjustment of parameters, and others were performed for model verification. The simulations included 1) a prolonged increase in dopamine concentration, replicating bath application [18,21,22]; 2) a prolonged increase in intracellular calcium concentration, replicating bath application of NMDA and AMPA agonists [16,17]; and 3) a prolonged increase in both dopamine and calcium concentration, replicating the study of Snyder et al. [29].

A simulated increase in dopamine from 10 nM (the basal level [24]) to 10 μM increases the levels of phosphoThr34 9-fold within 2 min, comparable with the experimental observation of a 6–9-fold increase within 2 to 4 min [17] (Figure 2A1, solid line). In parallel, simulated phosphoThr75 decreases to 60% of basal (Figure 2A2, solid line), in

| BIOCHEMICAL NETWORK |
|--------------------|
| Glutamate (NMDA) |
| Ca, CaM |
| CaMKII |
| PP2B (calcineurin) |
| PP1 |
| DARPP-32 |
| PKA |
| pThr34 |
| pThr75 |
| D2 |
| D1 |
| AC5 |
| PDE4 |
| PDE1 |
| PP2A |
| Cdk5 |
| MSN spine membrane |

Figure 1. Second Messenger Pathways Involved in the Phosphorylation of DARPP-32 on Thr34 and Thr75 in Medium Spiny Projection Neurons

A calcium elevation produced by glutamate leads to calcium binding to CaM (Ca,CaM), which activates both CaMKII and PP2B, the latter dephosphorylating DARPP-32 on Thr34. Stimulation of the dopamine D2 receptor activates the PKA cascade via ACS and CaM formation, PKA in its turn increases phosphorylation of DARPP-32 on Thr34, which then inhibits PP1. DOI: 10.1371/journal.pcbi.0020119.g001
This is in accordance with published data [15]. The mechanism behind the downregulation of phosphoThr75 by dopamine is not known; however, evidence suggests that dopamine acts by increasing the dephosphorylation of phosphoThr75 by PP2A [17]. Other experiments show that enhanced activation of PP2A via depletion causes an increase in phosphoThr75 [26].

A sustained increase in intracellular calcium concentration, as results from prolonged activation of NMDA or voltage-dependent calcium channels, leads to a decrease in the levels of phosphoThr34. In response to a 300-nM calcium signal, both phosphoThr34 and phosphoThr75 decrease to approximately half the basal level within 1 min (Figure 2B, solid lines), in agreement with experimental data [16,17,27]. The decrease in phosphoThr34 is due to the calcium-dependent activation of PP2B. In contrast, the dephosphorylation of Thr75 is dependent on calcium activation of PP2A. If calcium activation of PP2A is eliminated, a calcium elevation does not affect the levels of phosphoThr34 much (Figure 2B, dotted line). It is important to note that this modulation of PP2A by calcium has a minimal effect on the level of phosphoThr34 (Figure 2A1 and 2B1, dotted lines).

The model is verified by evaluating the change in phosphoThr75 and phosphoThr34 in response to paired dopamine and calcium elevations. As demonstrated experimentally [23], the calcium increase inhibits the increase in phosphoThr34 caused by dopamine (Figure 3A, solid line), and enhances the decrease in phosphoThr75 (Figure 3B, solid line). The decrease in phosphoThr34 caused by elevated calcium is due to PP2B both in the model (Figure 3, dashed lines) and experimentally. If the level of PP2B is decreased to 10% of control (simulating the effect of cyclosporin A for 20 min [27]), the effect of calcium on Thr34 phosphorylation is eliminated.

The steady-state experiments of either dopamine stimulation or calcium stimulation provided values for unconstrained rate constants; however, no rate constants in the model were adjusted while simulating the experiments with paired dopamine and calcium inputs. Thus, comparison of these simulations with experiments represents an authentic verification of the model. Taken together, these results

### Table 2. Reactions and Rate Constants of Ca to CaMKII and PP2B Pathway

| Reaction Equation | $k_d$(mM$^{-1}$sec$^{-1}$) | $k_b$(sec$^{-1}$) | $k_m$(sec$^{-1}$) |
|------------------|--------------------------|-----------------|-----------------|
| Ca + 2Ca$^2+$   | $6.00 \times 10^{-3}$    | 9.1             |                 |
| Ca$^2+$ + 2Ca$^2+$ | 0.1                      | $10^3$           | 0.1             |
| Ca$^2+$ + PP2B$^2-$ | PP2B$^2-$CaM$^2+$ | 1               | 3               |
| PP2B$^2-$CaM$^2+$ | $6.00 \times 10^{-3}$   | 0.91            |                 |
| PP2B$^2-$CaM$^2+$ | 0.1                      | 10              |                 |
| Ca$^2+$ + PP2B$^2-$ | PP2B$^2-$CaM$^2+$ | 1               | 0.3             |
| Ca$^2+$ + PP2B$^2-$ | PP2B$^2-$CaM$^2+$ | 1               | 0.3             |
| Ca$^2+$ + CaMKII | CaMKII$^2-$CaM$^2+$ | $7.50 \times 10^{-4}$ | 0.1 |
| CaMKII$^2-$CaM$^2+$ | PP1                      | $5.00 \times 10^{-3}$ | $3.00 \times 10^{-6}$ |
| CaMKII$^2-$CaM$^2+$ + PP1 |                 |                 |                 |

*Unit in sec$^{-1}$.  
**Unit in mM$^{-1}$sec$^{-1}$. 
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confirm that our model, based on available biochemical data from many independent studies of signalling pathways in the striatum, reproduces the changes in the phosphorylation levels of DARPP-32 at Thr34 and Thr75 produced by activation of dopamine-regulated second messenger pathways and changes in intracellular calcium concentration.

Response to Transient Inputs Differs from Response to Steady-State Inputs

In vivo, physiological elevations in calcium and dopamine are transient. Dopamine is transiently elevated subsequent to burst firing of nigral or ventral tegmental neurons in response to reward or expectation of reward [3]. Calcium is transiently elevated during striatal up-states [28–30], which are caused by periods of high frequency cortical glutamatergic inputs [31,32]. Since the changes in concentration of most signalling molecules are impossible to measure with this high time-resolution, computer simulations may help elucidate the function of complex second messenger pathways. Thus, the verified model is used to investigate the change in phophoThr34 and phosphoThr75 in response to transient inputs.

A brief dopamine pulse (Figure 4A, solid line; peak = 1 μM, half-width = 0.6 s), comparable to release evoked by a dopamine neuron burst [24,33,34] leads to a small, but sustained increase in free catalytic PKA (PKAc) (Figure 4A3) and a slightly longer increase in phosphoThr34 (Figure 4A4). If the same amount of dopamine is presented as a slow, low concentration signal (peak = 100 nM, half-width = 7 s; Figure

![Figure 2. Model Response to Either D1 Activation or Calcium Elevation](image)

Dopamine elevation at time 300 s (A3) leads to a nine times increase in phosphoThr34 within 2 min (A1, solid line), while phosphoThr75 is reduced to 60% of control (A2, solid line). This is quantitatively in accordance with experimental results. The PKA-dependent activation of PP2A is critical for this behavior; if left out of the model, phosphoThr75 increases instead of decreases (blue dashed line). Calcium-dependent activation of PP2A is not critical to simulate the correct response to D1 activation (purple dotted line). Calcium elevation at time 300 s (B3) leads to a reduction in both Thr34 (B1, solid line) and Thr75 phosphorylation (B2, solid line). In contrast to the result in A1 and A2, this response requires calcium-dependent activation of PP2A; if left out of the model (purple dotted line), calcium causes an increase in Thr75 phosphorylation.

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### Table 3: Reactions and Rate Constants of Phosphor/Dephosphorylation of DARPP-32

| Reaction Equation | $k_i(M^{-1}sec^{-1})$ | $k_o(sec^{-1})$ | $k_{rec}(sec^{-1})$ |
|-------------------|----------------------|-----------------|---------------------|
| DARPP32 + PKA+$\rightarrow$ DARPP32PKA+$\rightarrow$ Thr34 + PKA | $2.7 \times 10^{-3}$ | 8 | 2 |
| PP2A + PKA+$\rightarrow$ PKAP2A+$\rightarrow$ P2AP2A | $2.5 \times 10^{-3}$ | 0.3 | 0.1 |
| P2AP2A+$\rightarrow$ P2A | $4.0 \times 10^{-2}$ | 0.58 | 0.5 |
| $\mu$Thr34 + P1+$\rightarrow$ Thr34P1 | $1.0 \times 10^{-3}$ | 2 | 0.5 |
| Thr34P1 + PP2A+$\rightarrow$ Thr34PP2A+$\rightarrow$ DARPP32 + PP2A | $1.0 \times 10^{-3}$ | 2 | 0.5 |
| Thr34 + PP2A+$\rightarrow$ Thr34PP2A+$\rightarrow$ DARPP32 + PP2A | $1.0 \times 10^{-3}$ | 2 | 0.5 |
| DARPP32 + Cdk5+$\rightarrow$ DARPP32Cdk5+$\rightarrow$ Thr75 + Cdk5 | $4.5 \times 10^{-4}$ | 2 | 0.5 |
| Thr75 + PKA+$\rightarrow$ Thr75PKA | $3.7 \times 10^{-4}$ | 1 | 1 |
| Thr75 + PP2A+$\rightarrow$ Thr75PP2A+$\rightarrow$ DARPP32 + PP2A | $4.0 \times 10^{-4}$ | 12 | 3 |
| Thr75 + PP2A+$\rightarrow$ Thr75PP2A+$\rightarrow$ DARPP32 + PP2A | $4.0 \times 10^{-4}$ | 12 | 3 |
| PP2A + 4Ca+$\rightarrow$ PP2A | $7.72 \times 10^{-12}$ | $1.0 \times 10^{-2}$ | 1.6 |

$^a$Unit in sec$^{-1}$

$^b$Unit in M$^{-1}$sec$^{-1}$

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### Table 4: Molecule Quantities

| Molecule       | Quantity | Reference |
|----------------|----------|-----------|
| D$_2$R         | 500 nM   |           |
| G prot         | 3 μM     |           |
| AC5            | 2.5 μM   |           |
| ATP            | 2 mM     |           |
| PDE1           | 4 μM     |           |
| PDE4           | 2 μM     |           |
| PKA            | 12 μM    | [111]     |
| CaM            | 10 μM    | [112,113] |
| CaMKII         | 20 μM    |           |
| DARPP-32       | 50 μM    | [37]      |
| PP2A           | 2 μM     | [114,115] |
| PP2B           | 4 μM     |           |
| PP1            | 5 μM     | [116,117] |
| Cdk5           | 1.8 μM   |           |

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4A1, dashed line), the total amount of cAMP production is similar to that for the brief, high concentration input, although with a different time course that matches the dopamine signal (Figure 4A2). The changes in PKAc level (Figure 4A3) and in phosphoThr34 (Figure 4A4) are almost identical in the brief, high concentration dopamine input: not only are the amounts of free catalytic PKA and phosphoThr34 quite similar, but also the time courses are the same. Thus, PKAc activation acts as a temporal integrator of the cAMP signal.

A transient calcium elevation produces changes in several downstream enzymes (Figure 4B). PP2B is activated by Ca/CaM and dephosphorylates DARPP-32 at Thr34. This is the dominant effect of a slow, low concentration calcium signal (peak = 0.2 μM, half with 5 s; Figure 4B1, dashed line), leading to the expected and previously described decrease in Thr34 phosphorylation (Figure 4B4, dashed line). In addition, Ca/CaM also activates PDE1, and inhibits adenylate cyclase; both of these factors contribute to reduction of cAMP below basal levels (Figure 4B2). Though the decrease in cAMP concentration is expected to decrease the level of PKAc, this...
PKAc interaction with phosphoThr75 is important. The calcium-dependent decrease in the cAMP formation, but also the total formation of activated PKA (both free and bound to calcium) is transiently elevated, calcium-dependent activation of PP2A input due to the PKA dependent activation of PP2A. When in addition inputs are paired.

Some dephosphorylation on Thr75 occurs following a dopamine stimulus due to the PKA dependent activation of PP2A. When a fast calcium pulse (as illustrated in Figure 4B1) is given with each dopamine elevation, as would happen with simultaneous release from the glutamatergic and dopaminergic terminals, the activation of PKA is greatly enhanced compared with the response to dopamine alone. The increase in free PKAc due to paired stimuli is 40% greater than the increase observed with dopamine alone, whereas the peak increase in phosphoThr34 is more than doubled (Figure 5A and 5B, solid red lines). The increase in Thr34 phosphorylation in its turn produces an elevation in PKAc comparable with a positive ISI (Figure 5A, green dashed line), and significantly increases the phosphorylation of DARPP-32 at Thr34 (Figure 5B). When a fast calcium pulse (as illustrated in Figure 4B1) is given with each dopamine elevation, as would happen with simultaneous release from the glutamatergic and dopaminergic terminals, the activation of PKA is greatly enhanced compared with the response to dopamine alone. The increase in free PKAc due to paired stimuli is 40% greater than the increase observed with dopamine alone, whereas the peak increase in phosphoThr34 is more than doubled (Figure 5A and 5B, solid red lines). The increase in Thr34 phosphorylation in its turn decreases PP1 (Figure 5C), and thus the ratio of PKAc to PP1 is greatly enhanced (Figure 5D) compared with dopamine alone. Since the phosphorylation state depends on the balance between kinases and phosphatases, the increase in PKAc to PP1 is likely to produce a significant change in several target molecules, as has been demonstrated for AMPA receptors [35].

This calcium enhancement of the response to dopamine is in contrast to the effect seen with steady-state inputs in which steady-state calcium inhibits the steady-state dopamine response (compare Figure 3). The calcium pulse given simultaneously with the dopamine input also significantly enhances the decrease in phosphoThr75 (Figure 5E) due to the additional calcium-dependent activation of PP2A, which further elevates PP2A activity. This reduction in phosphoThr75, due to transient dopamine alone, is much smaller than the reduction observed with the high-amplitude (10 μM) steady-state dopamine input used in some experiments (compare Figure 2A2).

In reinforcement learning paradigms, the reward is given after the visual and motor stimuli; thus the dopamine stimulus occurs after the glutamate stimulus. If the reward occurs prior to the visual and motor stimuli, the animal does not learn. Thus, if the second messenger pathways in the model completely explain synaptic plasticity underlying reward learning, the model should be sensitive to interstimulus interval (ISI). Simulations were performed with the dopamine signal occurring prior to calcium (negative ISI) and after the calcium pulse (positive ISI). Large negative ISIs produce an elevation in PKAc comparable with a positive ISI (unpublished data), which is not consistent with behavior. Thus, the model’s sensitivity to ISI does not match that of the behavior. This leads to the prediction that although the model reproduces the cooperativity between glutamate and dopamine, additional mechanisms are required to provide temporal sensitivity.
Despite the larger increase in free PKAc with combined calcium and dopamine inputs, the total amount of PKAc produced (both free and bound to phosphoThr75) is still larger with dopamine alone (Figure 5F). That less PKAc becomes activated in the presence of increased levels of calcium may be expected because of the calcium-dependent AC5 inhibition as well as calcium-dependent PDE1 activation. Free PKAc levels increase following combined brief calcium and dopamine inputs because less PKAc becomes bound to phosphoThr75. This is further explained by the presence of the PKA–PP2A–phosphoThr75 loop.

Feedback Loop Modulates Dopamine-Stimulated PKAc and phosphoThr34

PKAc enhancement of PP2A activity (reaction (i) in Figure 6E); PP2A dephosphorylation of phosphoThr75 (reaction (ii)); and subsequent decreased inhibition of PKAc (reaction (iii)) can act on PKAc as a positive feedback loop. This loop is believed to enhance the levels of free PKAc, which would then enhance phosphorylation of DARPP-32 on Thr34 in response to dopamine stimulation [13]. To test this hypothesis, model simulations, using the same dopamine and calcium pulses as described above, are repeated with this positive feedback loop opened, by setting to zero the rate constants for reactions (i) and (iii). Removing the phosphorylation of PP2A by PKAc should decrease the modulation of phosphoThr75, and reduce the disinhibition of PKAc.

Surprisingly, opening the feedback loop increases the concentration of both PKAc (Figure 6A1, orange line) and phosphoThr34 (Figure 6A2, orange line) compared with the control condition (Figure 6A, black lines). Eliminating just the phosphorylation of PP2A by PKAc (reaction (i), magenta lines), or eliminating just the inhibition of PKA by phosphoThr75 (reaction (iii), light blue lines), produces a small increase in PKAc, but eliminating both reactions produces a dramatically larger increase in PKAc. The supralinear effect of these two feedback loop reactions suggest that the apparent inhibitory effect of the loop following a transient dopamine input is due to a change in equilibrium in the reactions involving PKAc. As more PKAc is formed, much of it is bound to PP2A in the enzyme-substrate complex, as well as to phosphoThr75 in the inhibitory complex (Figure S1A). Thus these two forms of bound PKAc act as a sink for free PKAc, dampening the stimulatory effect of dopamine on the increase in the free PKAc levels.

Eight transient calcium pulses cause an increase in both PKAc and phosphoThr34 (Figure 6B, black lines) that is comparable to the increase caused with eight pulses of dopamine alone (compare Figure 6A). Because calcium increases PP2A activity, it is possible that the feedback loop is involved in this response. Simulations show that when the feedback loop is partly or completely opened (Figure 6B), the response of PKAc and phosphoThr34 to a brief calcium input is dramatically reduced. Opening up both reactions in the loop actually transforms the effect of calcium on PKAc into inhibition (due to inhibition of AC5 and activation of PDE1).
Furthermore, the calcium-activated form of PP2A (PP2Ac) is line).

The decrease in phosphoThr34 due to the slow, low-amplitude signal is paired with a dopamine input alone (green line). A decrease of phosphoThr34 is seen following a calcium input alone (blue line).

The cumulative increase of free PKAc following eight high-amplitude, brief dopamine inputs (green line) is a bit larger than if a slow, low-amplitude calcium signal is paired with a dopamine input alone (blue line). Also the slower, low-amplitude calcium input alone (blue line) does not give any significant increase in PKAc.

Pairing slow, low-amplitude calcium inputs with dopamine inputs (red line) reduces the cumulative increase in phosphoThr34 compared with a dopamine input alone (green line). A decrease of phosphoThr34 is seen following a calcium input alone (blue line).

The decrease in phosphoThr34 due to the slow, low-amplitude calcium input is due to a prolonged activation of PP2B (dashed line) compared with when a brief, high-amplitude calcium input is given (solid line).

Furthermore, the calcium-activated form of PP2A (PP2Ac) is significantly less activated following a small but slower calcium input (dashed line) than if a high-amplitude calcium input is given (solid line). DOI: 10.1371/journal.pcbi.0020119.g007

Thus, binding of calcium to PP2A has two important effects. First, it reduces the amount of unbound PP2A, and thus reduces the substrate available for PKAc binding. This decrease in PKAc–PP2A (Figure 5B) shifts the equilibrium of reaction (i), thus PKAc dissociates from PP2A. Second, the enhanced PP2Ac activity dephosphorylates phosphoThr75 and disinhibits PKAc. The quantity of PKAc–phosphoThr75 decreases (Figure 5B), reflecting a shift in the equilibrium of reaction (iii), as PKAc dissociates from phosphoThr75. The stimulatory effect of calcium on PKAc requires the activation of PP2Ac, dephosphorylation of Thr75, and disinhibition of PKAc. Opening reaction (i) eliminates the reduction of PKAc–PP2A by calcium, and opening reaction (iii) eliminates the reduction of PKAc–phosphoThr75 by calcium.

When brief dopamine and calcium pulses are given together (eight paired pulses at 20-s intervals), the calcium-dependent enhancement through the PKA–PP2A–phosphoThr75 loop dominates (Figure 6C). The combination of dopamine and calcium produces a smaller quantity of the PKAc–PP2A complex than dopamine alone (Figure 5C). When reaction (i) and (iii) are set to zero, the reduction in PKAc–PP2A and PKAc–phosphoThr75 complexes are smaller than in the control case. Thus, when rates of reaction (i) and (iii) are set to zero (opening the loop), both PKAc and phosphoThr34 increase less compared with when the loop is fully active.

In the Thr to Ala mutant mouse [36], in which Thr75 cannot be phosphorylated, increasing dopamine levels no longer affect downstream processes such as CREB phosphorylation. To replicate this experiment, phosphoThr75 is set to zero in the model. The new equilibrium has a different basal level of PKAc and phosphoThr34 (unpublished data); however, the increase in PKAc due to paired stimuli is similar to the case when reaction (iii) is eliminated, consistent with experimental results [36,37]. In summary, the PKA–PP2A–phosphoThr75 feedback loop is important for increasing levels of free PKAc and phosphoThr34, but only when transient calcium elevations are present, and not in response to transient inputs of dopamine alone.

Calcium activation of the feedback loop is essential for the enhancement of PKAc and phosphoThr34 in response to paired stimuli. This implies that if calcium-dependent activation of PP2A is eliminated, but the PKA–PP2A–phosphoThr75 loop is kept intact, pairing calcium and dopamine will give smaller elevations of free PKAc and phosphoThr34, than dopamine alone. Furthermore, calcium stimulation alone will reduce both PKAc and phosphoThr34. This is partly due to calcium-dependent inhibition of AC5 and calcium-dependent activation of PDE1, but also because now the PKA–PP2A–phosphoThr75 loop instead works as a sink for PKAc. This prediction is tested with simulations in which the calcium-dependent enhancement of PP2A is eliminated (rate constants set to zero). Figure 6D shows that now calcium alone (blue lines) produces a decrease in both PKAc and phosphoThr34 (qualitatively similar to that observed with the feedback loop eliminated), and calcium paired with dopamine inhibits the production of PKAc and phosphoThr34 (red lines), again similar to that observed with the feedback loop eliminated. Thus, without the calcium-dependent PP2A augmentation, a transient dopamine input alone is predicted to increase both PKAc activation and phosphoThr34 more effectively than dopamine paired with calcium.

Role of Calcium Dynamics

Many different mechanisms control calcium dynamics in spiny projections neurons. Brief calcium elevations are produced by synaptic activation and backward propagating action potentials. A slower and lower calcium elevation might result from calcium release from intracellular stores. This is one proposed mechanism by which the postsynaptic dopamine D2 receptor acts [38]. Therefore, to evaluate the effect of calcium dynamics, simulations were repeated using a slower and lower calcium elevation (as in Figure 4B1, dashed line). When slow calcium inputs are paired with an increase in cAMP, the buildup of free PKAc is unchanged or minimally decreased compared with cAMP increase alone (Figure 7A), instead of enhanced as when faster, larger calcium pulses are used (compare Figure 5). In addition, the buildup of phosphoThr34 is decreased (Figure 7B).

These differences between a brief, high-amplitude calcium input and a slower, low-amplitude calcium input are due to the different effects that these calcium signals have on the level of PP2B and PP2A activation. Both the slow and the fast calcium inputs activate PP2B, though with a more prolonged time course if a longer calcium elevation occurs (Figure 7C, dashed line). But only the briefer and high concentration calcium activates PP2A significantly (Figure 7D, solid line).
The slow calcium pulse therefore does not enhance dephosphorylation at Thr75, and thus does not enhance PKAc.

The Main Simulation Results Are Independent of Model Parameters

The main findings from these simulations suggest that the presence of the PKA–PP2A–phosphoThr75 loop causes increased formation of free PKAc as well as phosphoThr34 if a dopamine input is paired with a transient calcium elevation. To investigate the robustness of this result, simulations are repeated with variations in the least constrained parameters. Though most rate constants are constrained by direct measurements of affinity, the dissociation constants of some reactions have not been reported. In particular, the dissociation rate for calcium binding to AC5, calcium–calmodulin binding to PDE1, and calcium-dependent activation of PP2A is not known. Thus, simulations are repeated using a range of dissociation rate constants for each of these reactions. Figure 8A and 8B shows that, over a large range of dissociation rates for calcium binding to AC5 or Ca4CaM binding to PDE1, the PKAc elevation and PP1 suppression in response to a dopamine signal is enhanced when dopamine is paired with calcium. The model is mildly sensitive to the rate of calcium-dependent enhancement of PP2A activity. If the activation and deactivation kinetics are much faster (100× as fast as control values), paired dopamine and calcium inputs are no more effective than dopamine alone (Figure 8C). With a fast dissociation rate, the deactivation of the calcium-activated PP2A (PP2Ac) occurs too rapidly to allow PP2Ac to dephosphorylate phosphoThr75 during brief calcium elevations. Also, if the calcium activation is very slow, then very little calcium-dependent activation of PP2A occurs during the brief duration of the calcium inputs, and the effect of paired stimulation decreases. A 100× slower kinetics of calcium-induced PP2A stimulation is, however, not in accordance with results from Nishi et al. [17], in which a significant dephosphorylation of phosphoThr75 occurs within 5 min.

Though the quantity of DARPP-32 has been estimated [37], enzyme quantities are typically measured from tissue volumes which include both neurons and non-neuronal cells. The actions of anchoring proteins for molecules such as PKA suggest that the effective quantities of these enzymes in the synapse may be much higher than published estimates [39]. Thus, simulations are repeated using both higher and lower quantities of the enzymes (PKA, cdk5, PP2A, PP2B) to determine to what extent the results are sensitive to these parameters. Figure 9A1 shows that, even with a 10-fold increase or decrease in enzyme quantity, calcium paired with dopamine produces a larger PKAc-to-PP1 ratio than dopamine alone. Simulations in which the quantity of single enzymes or pairs of enzymes was changed did not appreciably change the results. Since the ratio PKAc:PP1 controls AMPA channel phosphorylation, this larger increase in PKAc:PP1 would translate into more LTP due to paired stimulation.

Nonetheless, the signalling network exhibits changes in activity due to the change in enzyme quantity. Specifically, both the basal and stimulated levels of PKAc are altered. Figure 9A2 (dashed lines) shows that both basal and stimulated PKAc decrease when enzyme quantities are decreased to 10% of the control values, and that both basal and stimulated PKAc increase when enzyme quantities are increased. In contrast, the basal and stimulated levels of phosphoThr34 increase with a decrease in enzyme concentration (Figure 9A2, dotted lines). In summary, though the basal level of PKAc:PP1 changes with a change in enzyme quantity, the basic principle, that paired stimulation produ-
ces a larger increase than either calcium or dopamine alone, is robust to enzyme quantity.

To further demonstrate the importance of DARPP-32 in the integration of signalling pathways, simulations were repeated with parameter variations designed to make DARPP-32 independent of its regulatory molecules. The total concentration of PKA, PP2A, PP2B, and cdk5 were set to 10% of control values, and the dynamics of the PKA system were accelerated, i.e., the rate constants for all reactions involving PKA were increased 100-fold. These changes decreased the signal integration capability of PKA, and exposed the signal integration capability of DARPP-32.

As seen in Figure 9B1, the amplitude of the PKAc response increases since it is activated more efficiently by cAMP. In addition, PKAc decreases almost to basal level between each pair of dopamine pulses. In contrast, phosphoThr34 accumulates during the eight pulse stimulus (Figure 9B2), just as in the control model. More importantly, though calcium paired with dopamine produces a slight decrease in free PKAc, it produces an increase in phosphoThr34 (Figure 9B2). Thus, independent of the ability of PKAc to integrate dopamine and calcium signals, phosphoThr34 also integrates dopamine and calcium signals.

Last, we evaluated whether the results were contingent upon the high DARPP-32 concentration. Reducing DARPP-32 to 10\(\times\)molar produced a change in the basal level of PKAc, phosphoThr34, and PP1, but produced very little change in the ratio PKAc:PP1. More importantly, the increase in PKAc:PP1 in response to paired stimulation remains greater than the response to dopamine alone (Figure S2A), similar to simulations in which other enzymes were increased. The relative increase due to paired stimulation compared with the sum of dopamine and calcium inputs alone is larger with the higher DARPP-32 concentration compared with the lower, suggesting that the high concentration of DARPP-32 in striatal neurons that has been measured may have a physiological relevance. Nonetheless, these simulations further confirm the robustness to parameter variations of the main result, namely that combined transient inputs are more effective.

**Discussion**

To better understand the complex intracellular signalling networks underlying synaptic plasticity and reinforcement learning, we have developed a model of the calcium and cAMP signalling cascades that regulate DARPP-32 phosphorylation. The model is based on published biochemical data: the simulated regulation of DARPP-32 phosphorylation by sustained G-protein receptor activation and calcium influx fits experimental data, increasing and decreasing phosphoThr34, respectively (Figures 2 and 3).

Interestingly, our model suggests that the effect of transient calcium influx is very different from sustained...
The Role of DARPP-32 in Reinforcement Learning and LTP

The concomitant increases in calcium and dopamine are behaviourally relevant stimuli in that they occur during reinforcement learning. Visual stimuli and motor action cause corticostriatal fibers to release glutamate; the subsequent reward is accompanied by dopamine release from nigrostriatal fibers. Thus, it is possible that during reinforcement learning, the calcium elevation produced by glutamate stimulation, together with cAMP produced by dopamine stimulation, induces synaptic plasticity, which underlies learning the association between the visual stimulus and reward.

Theoretical models of reinforcement learning, e.g., temporal difference learning [8,46,47], posit that the striatum learns which among multiple actions produces the greatest future rewards. Memories of rewarded motor actions may be stored as potentiated corticostriatal synapses. During learning, those synapses that release glutamate when dopamine arrives are potentiated. Subsequently, glutamate stimulation alone evokes output from those neurons encoding the reinforced motor action.

Experimental support for temporal difference learning is rather strong. Experiments from striatal slices show that release of glutamate from corticostriatal fibers produces LTP in the presence of dopamine. Neither dopamine alone nor cortical stimulation alone produces LTP [48–50], and corticostriatal LTP is blocked in animals lacking DARPP-32 [49]. Activated PKA is known to mediate phosphorylation of Ser845 on the AMPA receptor GluR1 subunit [51,52], leading to insertion of AMPA receptors on the cell surface [45,53,54].

The model results presented here help explain the mechanisms underlying temporal difference learning. The prevailing view, based on biochemical experiments used to derive our model, has been that glutamate and dopamine have opposite effects on the PKA signalling cascade. Calcium decreases PKA activity, and increases PP1 activity, whereas dopamine does the opposite. In contrast, the model shows that transient calcium stimulation can enhance the PKA signalling cascade. Thus, when transient dopamine and glutamate are given together, the increase in the ratio of PKA to PP1 activity is enhanced compared with when dopamine is given alone (Figure 5). Thus, model simulations suggest that PKA activation, phosphorylation of DARPP-32 at Thr34, and subsequent PP1 inhibition, produced by conjunctive glutamate and D1 receptor stimulation, can produce LTP by increased phosphorylation of AMPA receptors. This suggests that the cellular mechanisms leading to temporal difference learning is the enhanced stimulation of PKA with paired calcium and dopamine stimuli. Thus, at a network level, when a subset of spiny projection neurons are activated by glutamate and dopamine simultaneously, corticostriatal synapses of those spiny projection neurons are strengthened; subsequently, glutamate stimulation alone evokes output from the correct neurons.

The dynamics of the calcium transient controls the effect of calcium on the dopamine induced PKA elevation. A persistent calcium elevation has a minimal effect on PKA and decreases phosphoThr34, whereas a brief transient calcium elevation produces an increase in both PKA and phosphoThr34. These results lend significance to the calcium imaging studies [29,30] which show that a backward propagating action potential enhances the calcium elevation during an up-state. This suggests that synaptic plasticity may require not only coincident glutamate and dopamine inputs, but also sufficient spiny projection neuron activity to produce a backward propagating action potential and the accompanying fast, high-amplitude calcium transient. This requirement for glutamate, dopamine, and a backward propagating action potential is the “three-factor rule” [7,55]. Nonetheless, the response to paired calcium and dopamine stimuli did not match the sensitivity to ISI as seen with behavior. Several other mechanisms within spiny projection neurons may be responsible for this sensitivity.

1) G protein coupled receptors such as mGluR and mAChR produce diacylglycerol and inositol triphosphate leading to calcium release from intracellular stores. Calcium release plays a role in sensitivity to ISI in cerebellar Purkinje cells [56,57]; and diacylglycerol activates protein kinase C, which releases calmodulin (CaM) from neurogranin [58]. When included in future versions of the medium spiny neuron model, these mechanisms may produce sensitivity to ISI.

2) In medium spiny neurons, various receptors and enzymes are localized to different compartments. For example, calcium influx through NMDA receptors occurs at the postsynaptic density, and dopamine receptors are distributed not only on the spines, but also on the dendritic shaft [59]. Anchoring of molecules such as CaMKII and PKA at the postsynaptic density [39,60] and diffuse distribution of other molecules such as PP2A implies that diffusion plays a role in the interaction between dopamine and calcium-activated pathways. This diffusion, by introducing temporal delays, may produce sensitivity to ISI. In addition to these intraneuronal mechanisms, it is possible that network interactions or presynaptic dopamine receptors may produce the ISI sensitivity observed behaviourally.

Transient and Sustained Calcium Have Different Effects on DARPP-32 Phosphorylation at Thr34

The nature of biochemical experiments makes it difficult to measure phosphorylation state with high time-resolution.
Thus, the simulation result that transient calcium enhances phosphoThr34 is a prediction of the model (Figures 4–6). Recently, Nishi et al. used glutamate for brief stimulations (15 s) and did see an increase in phosphorylation at Thr34 [61]. Although they suggest this increase is mediated indirectly, via release of nitrous oxide and production of cGMP, model simulations suggest that it can also be an effect of calcium increase through NMDA receptors.

The mechanism of action of D2 receptors in the striatum is unclear, since both inhibition of adenylate cyclase [69] and release of intracellular calcium [38,68] has been proposed. We show here that slow calcium transients, as occurs with calcium release from intracellular stores, are able to dephosphorylate Thr34. This may explain observations that activation of D2 receptors decreases phosphoThr75 and decreases the phosphorylation of PP1 substrates [21,64].

**The dual role of Thr75 phosphorylation.** The disinhibition of PKA when phosphoThr75 is dephosphorylated has been suggested to be an important part of the potentiating effect of DARPP-32 on PKA activity [65]: PKA enhances PP2A activity via phosphorylation; then PP2A dephosphorylates phosphoThr75 at an accelerated rate and the decrease in phosphoThr75 leads to more active PKA. Nonetheless, during transient dopamine inputs alone, the loop acts as a sink, in which binding of PKAc to PP2A or phosphoThr75 actually decreases the amount of free PKAc, and thereby decreases the ability of PKAc to phosphorylate DARPP-32 on Thr34. In contrast, the stimulation of PP2A through calcium shifts the balance of the loop, increasing dephosphorylation of phosphoThr75 and thus disinhibiting PKA. Figure 10 illustrates these two alternative modes of operation of this loop. The relative changes in the reaction flows are dependent on the biochemical reactions involving PKAc (Figure S1 further illustrates the quantitative role that PP2A substrate depletion and phosphoThr75 inhibition of free PKAc play in the feedback loop). An important difference between transient and steady-state calcium inputs is predicted by the model, due to the relative activity of PP2A versus PP2B. Steady-state calcium produces a large increase in PP2B, dephosphorylating phosphoThr34; in contrast, large, transient calcium elevations produce an increase in PP2A activation and consequent disinhibition of free PKAc due to phosphoThr75 dephosphorylation. The net effects of these different calcium inputs on the PKAc:PP1 balance are summarized in Figure S2B. If calcium is prevented from binding to and enhancing PP2A activity, then a transient calcium stimulation does not increase PKAc activity, but instead decreases PKAc. Similarly, if PP2Ac is prevented from dephosphorylating DARPP-32 on Thr75, then instead of an increase in PKAc activation following calcium stimulation, the amount of activated PKAc is decreased (compare Figure 6D).

One validation of the importance of phosphoThr75 in the action of calcium can be found in the study of different psychotomimetics in mice in which DARPP-32 has been genetically modified at either Thr34 or Thr75 so that these sites cannot be phosphorylated [36]. Phencyclidine (PCP), a drug that affects glutamate transmission and calcium influx, is the only drug whose effects are modified in the mice where Thr75 could not be phosphorylated, whereas lysergic acid diethylamide (LSD), which alters serotonergic transmission, and amphetamine, which alters dopamine transmission, have the usual effect on behavior in these animals.

**Regulation of Phosphatases Shape the Response of the Signalling Networks**

The regulation of kinases such as PKA and CaMKII has been studied in great detail, whereas much less attention has been given to the regulation of phosphatase activity. Nonetheless, our model, together with other modeling work [66–68], clearly suggests that the regulation of protein phosphatases is very important to shape the response in signalling networks. In the DARPP-32 signalling pathway, dynamic regulation of the level of phosphoThr75 by PP2A is essential. To reproduce experimental data on DARPP-32 phosphorylation, the regulation of PP2A by both calcium and dopamine is required. Although only PKA regulation has been directly proven, experiments show that glutamate inhibits phosphoThr75 [17] via a PP2A and calcium-dependent pathway. The importance of this pathway for mediating the cooperative action of calcium and dopamine suggests that
future experimental demonstration and kinetic characterization of calcium-activated PP2A is critical.

Results Are Robust to Variations in Parameters

A common criticism of this type of model is that simulation results are sensitive to parameters, and that insufficient data is available to constrain the parameters. To preempt this criticism, several simulations demonstrate that the present model is robust to variation in the unconstrained parameters.

The result that a transient calcium elevation can increase phosphoThr34 was shown with a previous model [69]. This earlier result was critically dependent on the presence of a Ca4CaM-stimulated form of AC; however, the predominant form of AC in striatum is type 5 [70,71], which is not stimulated by calcium. Incorporating the effect of phosphoThr75 and the regulation of PP2A in our model made the stimulatory effect of calcium robust to parameter variations (Figure 8).

A potential source of error in this model is the concentration of various components. Although the concentration of many of the enzymes has been published, the data usually comes from crude homogenates, and does not take into account compartmentalization or scaffolding of molecules. As shown in Figure 9, however, the most important conclusions from this model are qualitatively robust, even when enzyme concentrations are varied 10-fold.

Experiments have not shown directly whether the phosphorylation of Thr34 influences the rate of phosphorylation of Thr75. It is known, however, that phosphorylation of Ser 102 and Ser 137 influences the rate of phosphorylation of Thr34 [72,73], indicating that intramolecular mechanisms are important. Model simulations justified the constraint that DARPP-32 can only be phosphorylated at one threonine residue at a time. An alternative model, in which phosphorylation rate constants at one threonine site are lower (1/5) when the other threonine is phosphorylated gave very similar results (unpublished data). Nonetheless, this is an issue that needs to be addressed experimentally.

In conclusion, our results support the idea that subcellular processing, such as coincidence detection, signal amplification, and decision making, based on the interactions within the intracellular networks, are essential for control of neuronal activity [74]. Some of these critical interactions control whether learning and synaptic plasticity will occur in different brain regions (see, e.g., [57,75]). Since it is exceedingly difficult to visualize the dynamics of intracellular signalling pathways, modeling is an important adjunct method for formulating hypotheses regarding the critical steps in these pathways [69,76]. New imaging techniques such as Fluorescence Resonance Energy Transfer (FRET) [77,78], rather than removing the need for dynamic modeling, instead provide essential constraints that improve the veracity of such models. Future modeling approaches that incorporate both this data and compartmentalization of enzymes due to anchoring proteins [35,79–81] will have an increased ability to formulate hypotheses regarding key molecules controlling neuronal dynamics and plasticity in the basal ganglia.

Materials and Methods

All reactions in the model are described as protein–protein interactions:

or as enzymatic reactions written in the Michaelis–Menten form which assumes a nonreversible catalytic step:

\[ A \rightarrow \frac{k_{\text{cat}}}{k_{\text{in}}}} \]

\[ A + E \rightarrow \frac{k_{\text{cat}}}{k_{\text{in}}} A + E \]

The concentration of the substrate A in reaction 1 is described using a first-order differential equation of the form:

\[ \frac{d[A]}{dt} = k_{\text{on}} [A] - k_{\text{off}} [A][B] \]

Solution of this equation requires initial concentrations and the forward (k_{\text{on}}) and backward (k_{\text{off}}) rate constants for reaction N. For cascades of reactions, equations are derived by summing terms describing all reaction pathways leading toward or away from a particular molecule. For example, the rate of change of concentration of AC in Equation 2 is given by:

\[ \frac{d[AE]}{dt} = k_{\text{on}} [A][E] - k_{\text{off}}[AE] - k_{\text{decat}}[AE] \]

[76,82]. For protein–protein interactions, the equilibrium constant, K_d, is defined as the ratio of backward-to-forward rate constant: k_{\text{off}}/k_{\text{on}}. For enzymatic reactions, k_{\text{cat}}/k_{\text{in}} defining the last, catalytic step, is the rate at which product appears (sometimes called V_max), and the affinity, K_M = (k_{\text{cat}} + k_{\text{in}})/k_{\text{cat}}. When k_{\text{cat}} is not known explicitly, k_{\text{cat}} is defined as 4 k_{\text{in}} [76,83]. As explained below, all parameters are derived from experimentally measured constants found in publications, or carefully inferred from indirect biochemical studies.

The reactions and rate constants in the model are summarized in Tables 1–3 and Figure 1. The total concentrations of molecules are summarized in Table 4. The equations were programmed in XPPAUT (http://www.math.pitt.edu/~bard/xpp/xpp.html) and run under the UNIX operating system. Simulations used the numerical integration method called “stiff” with a time step of 0.01–0.1 s.

Dopamine and the G-protein coupled receptor. The model has a tonic dopamine level of 10 nM, and stimulated or phasic dopamine pulses reach a concentration of 1 μM. The tonic dopamine maintains both the phosphoThr34 and phosphoThr75 at the basal level observed experimentally (via a PP2A-dependent mechanism, see below). In striatum the postsynaptic dopamine D1 type receptor (D1R) binds to AC5, and activates adenylate cyclase (see below), and also autohydrolyzes into GTP. The results do not differ if G_T is coupled to the G_TG type of GTP-binding protein [84]. The D1R can bind to either the inactive G protein first, and then dopamine, or dopamine first and then the inactive G protein [85–87]. Once the complex is formed, it rapidly dissociates into ligand-receptor complex, inactive G and subunit, and also autohydrolyzes into G, which then binds to G to regenerate inactive G protein. Compared to G, G binds to AC with a higher K_d and has a faster rate of hydrolysis [88].

Cyclic AMP formation and PKA activation. Active G binds to and activates adenylate cyclase type V (AC5) in the striatum [70,71,87], which produces cyclic AMP (cAMP). A simplified model of the AC5 enzyme reaction was created by combining several intermediate steps from a detailed AC model [89]. The rate constants for the simplified AC5 model were adjusted, using the nonlinear least-squares regression algorithm of Dynafit [90] (http://www.biokin.com/dynafit/). Figure S3 shows that the time course of cAMP production is almost identical to that of the Dessaur model for our conditions [89].

Calcium causes a 50% reduction in the activity of AC5 [91], though it is not known whether calcium binds to AC5 before or after binding to G. In the model, calcium is allowed to bind to the inactive AC5, which then can be activated by G/GTP. The results do not differ if calcium also is allowed to bind to active AC5. A step of regeneration of ATP is included that allows for a steady-state concentration of ATP in the absence of stimulation. ATP is only modeled explicitly in the formation of cAMP and not in any other enzymatic phosphorylation step below where it is not assumed to be a rate-limiting substrate.

Several types of phosphodiesterases (PDE) degrade cAMP in the striatum. The model includes the calcium-activated PDE1 [92] and a constitutively active form (referred to as PDE4) that represents PDE4B, PDE10A, and PDE7 [93–96].

cAMP binds to and activates PKA by binding to the regulatory
The dissociation rate of PP2B-CaM and PP2B-Ca_{2+}CaM is slower than for PP2B has a lower affinity for CaM than for Ca_{4+}CaM, and the calcium affinity of phosphoThr{\textsubscript{34}} for PP1 implies that almost all phosphorylation at Thr{\textsubscript{34}} occurs when calcium activates PP2A and thus decreases phosphoThr{\textsubscript{34}}. If the reaction is independent of calcium, the results do not change significantly, only unbound phosphoThr{\textsubscript{34}} can be dephosphorylated in the model.

Supporting Information

Figure S1. Binding of PKAc to phosphoThr{\textsubscript{75}} and PP2A Enzyme Complexes Following a Burst of Transient Calcium and Dopamine Inputs

Supplementary information related to Figure 6A–6C and Figure 10. The changes in PKAc–PP2A and PKAc–phosphoThr{\textsubscript{75}} complexes following (A) eight brief, high dopamine pulses, (B) eight brief, high calcium pulses, or (C) eight paired dopamine and calcium pulses while opening different parts of the PKA–PP2A–phosphoThr{\textsubscript{75}} feedback loop. The PKA complexes increase when dopamine is given alone, but not if a transient calcium input is provided. This decrease in the presence of a rapid calcium input occurs because calcium activates PP2A and thus decreases phosphoThr{\textsubscript{75}}. If the reaction is independent of calcium, the results do not change significantly, only unbound phosphoThr{\textsubscript{34}} can be dephosphorylated in the model.

Figure S2. Changes in the PKAc:PP1 Ratio

(A) Change in the PKAc:PP1 ratio when DARPP-32 concentration is decreased below control. In all cases a combined dopamine and calcium input produce a higher ratio than either input alone. (B) Effect of calcium dynamics on the PKAc:PP1 ratio. Complementary information to the results shown in Figure 5D. Eight fast, high calcium pulses increase the PKAc:PP1 ratio much more than eight slower and lower calcium pulses (as in Figure 4B, dashed lines). The red trace is the control trace from Figure 5D when brief dopamine and calcium inputs are paired. A steady-state calcium elevation which is large (2,000 nM, dashed line) or moderate (300 nM, dotted line) instead decreases the PKAc:PP1 ratio since CAMP production is decreased and PP2B-dependent dephosphorylation of phosphoThr{\textsubscript{34}} is enhanced.

Figure S3. Comparison between the Simplified, Reduced AC5 Model (Solid Line) and the Original Full Model (Dashed Line) by Dessauer et al.

The formation of cAMP (red) from 2 mM ATP and the reduction of cAMP (blue) show similar dynamics in both versions of the model.

Acknowledgments

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