Molecular effects of dopamine on striatal-projection pathways

Charles R. Gerfen

Gene regulation studies demonstrate that dopamine differentially regulates the direct and indirect projection neurons of the striatum through their respective expression of the D1 and D2 dopamine receptors. Induction of immediate–early genes (IEGs) in striatal neurons is used to study dopamine-receptor-mediated neuronal plasticity. In the dopamine-depleted striatum there is a switch in receptor-mediated signal transduction mechanisms to produce a supersensitive form of D1- mediated neuronal plasticity. This switch is suggested to underlie dopamine-agonist-induced dyskinetic movements that develop during the treatment of Parkinson's disease.

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THE NORMAL FUNCTION of the basal ganglia is dependent on the balanced activity of the two main output pathways of the striatum: the direct and indirect projection systems. Degeneration of the nigrostriatal dopamine pathway results in an imbalance in the activity of these pathways and is thought to be responsible for the movement disorders associated with Parkinson's disease (PD)[1,2]. Gene-regulation studies have demonstrated that this imbalance is a consequence of the segregation of the D1 and D2 dopamine-receptor subtypes on the direct and indirect striatal projection neurons[3]. Dopamine-receptor-agonist therapies are effective in reversing some of the effects of dopamine depletion. However, dopamine depletion also leads to the development of a supersensitive response of direct striatal neurons to D1-receptor agonists, as indicated by the induction of the expression of immediate–early genes (IEGs). In the normal striatum, D1-receptor-mediated IEG expression serves to limit neuronal plasticity, whereas in the dopamine-depleted striatum, D1-receptor stimulation results in an aberrant, persistent form of neuronal plasticity. This D1-receptor supersensitive response might be involved in the development of dyskinetic movements following prolonged treatment with dopamine-receptor agonists in PD.

The basal ganglia affects movement through outputs of the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr), which inhibit thalamocortical projections to the prefrontal, premotor and motor cortical areas responsible for the generation of purposive movements[2,4,5]. Current thinking has revolved around the notion that activity in the output pathways of the basal ganglia is determined by opposing excitatory and inhibitory inputs, as shown in Fig. 1. This theory holds that two distinct striatal projection pathways differentially contribute to excitatory and inhibitory circuits that regulate basal-ganglia output[6]. One pathway, the so-called direct striatal projection pathway, provides direct inhibition of the output nuclei of the basal ganglia. The other pathway, the indirect pathway, provides input to the circuit involving the external segment of the globus pallidus and subthalamic nucleus (STN)[7], which provides excitatory drive to the output nuclei of the basal ganglia[8].

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A model of basal-ganglia function was developed around the idea that activity in the direct and indirect pathway facilitates and inhibits movement respectively; imbalances in these pathways lead to movement disorders[1]. According to this model, in the resting condition, basal-ganglia output provides tonic inhibition to the thalamocortical system to inhibit movement. This tonic activity is produced by excitatory outputs of the STN, which are regulated by the indirect striatal pathway and a direct pathway from the cortex. By contrast, activity in the direct striatal projection pathway suppresses the tonic inhibitory output of the basal ganglia. Generation of movement during such pauses in basal-ganglia output activity has been well established for saccadic eye-movements[9] and limbs, although a similar correlation between pauses in activity of the output pathway and axial movements has not been demonstrated[10–12]. Thus, it is likely that imbalances in the normal activity of the direct and indirect striatal output pathways contribute to or even underlie the movement dysfunction in PD (Refs 2,13).

Gene-regulation studies have provided some additional insight into the function of dopamine within the striatum. These studies clearly demonstrate that dopamine differentially affects the function of striatal neurons that contribute to the direct and indirect pathways. In animal models of PD, striatal dopamine depletion leads to a permanent change in the response of striatal neurons to dopamine-receptor stimulation. Such a change in the functional activity of these striatal neurons might be responsible for the motor complications that accompany L-dopa treatment in PD.

Dopamine and the direct and indirect striatal projection systems

Segregation of the D1 and D2 receptors on the striatal neurons that give rise to the direct and indirect striatal projection pathways has been thought to be responsible for the opposing effects that dopamine exerts on these striatal output pathways[3]. Dopamine acts within the striatum predominantly through the D1- and D2-receptor subtypes, which have been estimated to comprise the vast majority of dopamine receptors within the striatum[14]. The opposing effects of dopamine mediated through these receptors are a result of the coupling

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of the D1 receptor to the stimulatory G proteins, G_s and G_{olf}, which stimulate activation of adenylate cyclase, and the coupling of D2 receptors to G proteins that inhibit adenylate cyclase[15,16].

The opposing effects of dopamine on the direct and indirect striatal neurons are evident from studies that have measured the expression of genes encoding the peptides that are co-expressed in these GABAergic medium spiny neurons[17,18] (Fig. 2). In the dopamine-depleted striatum, peptide levels in striatal neurons that give rise to the direct and indirect pathways are oppositely altered[3]. Thus, in direct striatal projection neurons, dopaminergic-neuron denervation results in decreased expression of substance P and dynorphin, owing to the absence of the stimulatory effects that dopamine exerts on these neurons through the D1 receptor. Conversely, in indirect striatal projection neurons, the absence of dopamine-mediated inhibition through the D2 receptor results in elevation of peptide markers such as enkephalin. In keeping with this concept, selective dopamine-receptor-agonist treatments are able to reverse the effects of dopamine depletion. Treatment with the D1-receptor selective agonist SKF38393 reverses the decrease in substance P in D1 neurons without affecting the levels of enkephalin in neurons that possess D2 receptors. Conversely, the D2-receptor selective agonist quinpirole reverses elevated enkephalin levels that result from dopamine depletion in striatal neurons that contain D2 receptors, without affecting the level of substance P in striatal neurons that contain D1 receptors. These changes in the levels of markers in the direct and indirect striatal neurons appear to reflect homeostatic responses of striatal neurons to the level of dopamine input within the striatum.

Opposing effects of dopamine on direct and indirect striatal projection neurons are also evident in the levels of expression of the constitutively expressed IEG *Egr1* (also known as *zif268*) following acute administration of D1- and D2-receptor agonists[19]. Treatment with D1-receptor agonists alone results in a selective increase in Egr1 levels in D1-receptor-bearing neurons. However, D2-receptor-agonist treatment administered either alone or in combination with a D1-receptor agonist results in the selective decrease in *Egr1* expression in D2-receptor-bearing neurons. Thus, when D1- and D2-receptor agonists are administered together, striatal output pathway neurons display opposite responses. This is reflected by increased expression of *Egr1* in D1-receptor-bearing neurons and decreased expression in D2-receptor-bearing neurons.

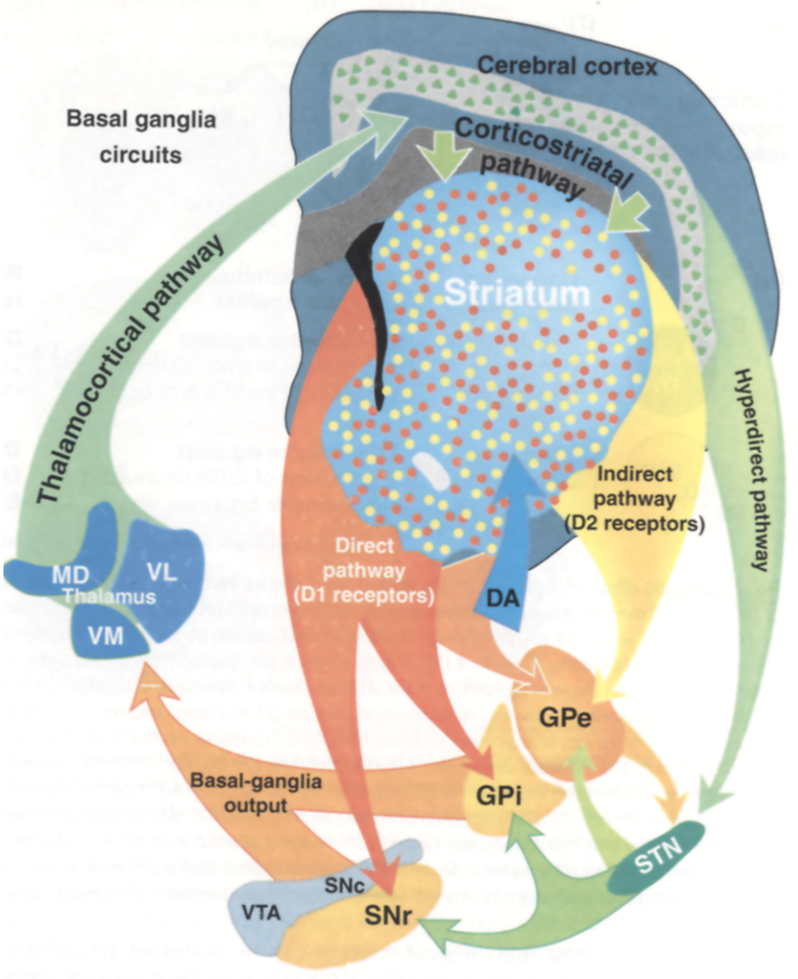

Fig. 1. ***Major neural circuits of the basal ganglia.*** *Excitatory projections are depicted as green arrows. Inhibitory projections are depicted as red, orange or yellow arrows. Cerebral cortical neurons in layer 5 provide excitatory, glutamatergic projections to the striatum (corticostriatal pathway). The striatum comprises two populations of medium spiny projection neurons. Direct-pathway neurons (red) bear D1 receptors and provide inputs directly to the output nuclei of the basal ganglia, the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr). Indirect-pathway neurons (yellow) possess D2 receptors and provide indirect projections to the output of the basal ganglia, through projections to the external segment of the globus pallidus (GPe). The GPe provides inhibitory inputs to the GPi and SNr (not shown). The GPe is also interconnected with the subthalamic nucleus (STN). The STN receives inputs directly from the cortex through the hyperdirect pathway and provides excitatory inputs to the output nuclei of the basal ganglia. The Gpi and SNr provide inhibitory inputs to thalamic nuclei, the mediodorsal (MD), ventromedial (VM) and ventrolateral (VL) nuclei, which project to prefrontal, premotor and motor cortical areas (thalamocortical pathway). Dopaminergic neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) give rise to dopamine projections to the striatum (DA, blue arrow).*

Dopamine depletion and the indirect striatal pathway

Reversal of the effects of striatal dopamine depletion on the altered levels of peptides described above is dependent on the mode of administration of dopamine-like agents[3]. Repeated single-dose administration of a D1-receptor agonist over several days reverses the lesion-induced decrease in substance P. However, reversal of the elevated levels of enkephalin in D2-receptor-bearing striatal neurons requires continuous stimulation with a D2-receptor agonist. Similarly, L-dopa treatment is effective at reversing decreased substance P levels, but ineffective at reversing elevated enkephalin levels when administered on an intermittent basis[20].

Parkinsonian motor features caused by the degeneration of the nigrostriatal dopamine system in PD have been suggested to result primarily from disinhibition of STN excitatory neurons, which results in an increase in the inhibitory output from basal ganglia to thalamus[2]. Although this model has been challenged as overly simplistic, it has several compelling features. First, as discussed above, dopamine depletion results in alterations in gene markers of striatal neurons that are consistent with this model[17,18]. Second, in primate models of PD, lesions of the STN reverse parkinsonian motor features[21]. Third, neurosurgical procedures that reduce activity in the STN or GPi have been shown to improve the motor features of PD (Ref. 13).

Based on the results of gene-regulation studies suggesting that motor dysfunction in PD is the result of a striatal dopamine-depletion-induced imbalance in the direct and indirect striatal output pathways, a rational

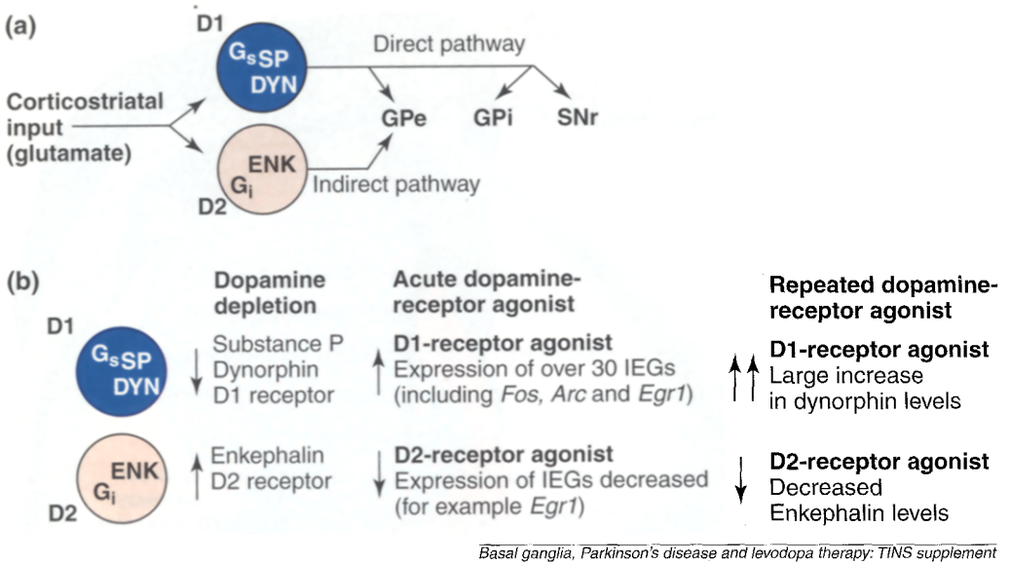

Fig. 2. ***Opposing effects of dopamine on the striatal direct and indirect pathway neurons are mediated by the segregation of D1 and D2 dopamine receptors on these neurons.*** **(a)** *Direct pathway neurons contain substance P (SP), dynorphin (DYN) and D1 receptors, which are coupled through the stimulatory G protein (G_s) to stimulate adenylate cyclase. Indirect pathway neurons possess enkephalin (ENK) and D2 receptors, which are coupled through the inhibitory G protein (G_i) to inhibit adenylate cyclase.* **(b)** *Dopamine depletion in the striatum results in decreased levels of SP, DYN and the D1 receptor in direct pathway neurons, owing to the loss of stimulation through D1 receptors, and results in increased levels of ENK and D2 receptors, owing to the loss of dopamine inhibition through D2 receptors. Acute D1-receptor agonist treatment results in the expression of over 30 immediate–early genes (IEGs) such as* Fos, Arc *and* Egr1 *in direct pathway neurons. Acute D2-receptor agonist treatment depresses expression of IEGs, such as the constitutively expressed IEG* Egr1, *in indirect pathway neurons. In the dopamine-depleted striatum repeated treatment with D1- and D2-receptor agonists result in opposite effects on gene expression in direct and indirect pathway neurons. D1-receptor agonists increase SP and DYN selectively in direct pathway neurons, whereas D2-receptor agonists decrease ENK levels in indirect striatal pathway neurons. Abbreviations: GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; SNr, substantia nigra pars reticulata.*

pharmacological strategy can be designed. In order to reverse the increased function in the indirect pathway, continuous or long-lasting activation of the D2 receptor with dopamine-receptor agonists would be expected to be effective[22]. Indeed, such agonists have been used in the treatment of PD (Refs 13,23,24) and provide antiparkinsonian benefits. However, it is generally necessary to supplement selective D2-receptor-agonist therapy with L-dopa in order to achieve maximal clinical effect. This might relate to the capacity of L-dopa to stimulate both D1- and D2-receptor-bearing neurons, which could be required for optimal effect. Thus, although D2-receptor-agonist treatments might be effective at normalizing function in the indirect striatal pathway, L-dopa might be required to activate the direct striatal output pathway to restore movement fully.

It is more difficult to restore normal function in D1-receptor-bearing, direct projecting striatal neurons. In animal studies, D1-receptor agonists or L-dopa are effective at reversing the decreased expression of substance P and dynorphin that results from dopamine deafferentation. However, repeated treatments result in an excessive increase in dynorphin. Moreover, as will be discussed below, following dopamine depletion in the striatum, the response of direct striatal output neurons to D1-receptor agonists becomes permanently supersensitive. This supersensitivity is reflected by the expression of IEGs in response to D1-receptor-agonist treatment.

IEG responses of striatal neurons

The acute response of striatal neurons to dopamine-receptor agonists can be measured by the induction of IEGs (Ref. 25). These genes constitute a growing number whose expression is induced in neurons under conditions that lead to neuronal plasticity[26]. Receptor-mediated signal-transduction mechanisms that result in the elevation of second messengers, including cAMP and Ca^{2+}, result in phosphorylation of transcription factors such as cAMP-response-element-binding protein (CREB) through activation of protein kinases. These transcription factors bind to promoter regions of IEGs, leading to their expression[27,28]. Many IEGs, such as *Fos*, *Egr1* and *Jun* encode transcription factors that, in turn, induce the expression of secondary response genes. In some cases, even such secondary genes are expressed quite rapidly and might therefore be classified as IEGs. Although many IEGs are transcription factors, others have a variety of functions. For example, the IEG *Arc* encodes a cytoskeletal protein[29] and the IEG *Homer* encodes a protein that binds to metabotropic glutamate receptors[30].

The induction of expression of IEGs occurs through a variety of receptor-mediated signal-transduction mechanisms[28]. The relationship between induction of IEGs and the physiology of neurons in which they are expressed remains unclear. Thus, it is probably inappropriate to equate the expression of IEGs to a particular level of neuronal activity. However, it is appropriate to view the expression of IEGs as indicating some change in the function of a neuron. In the striatum it would appear that such neuronal plasticity might have different functions. In some cases, IEG expression would appear to function as an adaptive response to stabilize the response of striatal neurons to extreme conditions of receptor activation. In other cases, IEG induction can result in activity-dependent enhancement of corticostriatal glutamate-receptor synapses.

Adaptive neuronal plasticity and D1-receptor stimulation

In the normal striatum, D1-receptor-mediated neuronal plasticity functions to stabilize the response of direct projecting striatal neurons[31,32]. This adaptive function can be demonstrated by studies of the effects of cocaine treatment, which results in excessive dopamine function, owing to the inhibition of dopamine reuptake[33,34]. Treatment with cocaine induces the expression of IEGs such as *Fos* and *Arc* in the dorsal striatum through activation of D1 receptors[35,36], which is restricted to direct projecting striatal neurons[37]. This occurs in a regional pattern that is inversely related to the normal distribution of the opioid peptide dynorphin[32]. Dynorphin is synthesized by D1-receptor-bearing striatal neurons throughout the striatum, but exhibits a significantly higher level of expression in the ventral striatum[18,38]. Opioid-receptor agonists infused into the dorsal striatum reduce the initial response to cocaine[35]. Moreover, repeated daily administration of cocaine results in increased synthesis of dynorphin in the dorsal striatal neurons that exhibit IEG expression on the first

and second day of cocaine treatment[32]. By the third day of cocaine treatment, D1-receptor-bearing neurons in the dorsal striatum produce high levels of dynorphin and the IEG response has diminished to near baseline levels. These studies indicate that excessive D1-receptor stimulation in the normal striatum results in the expression of IEGs. This leads to increased synthesis of dynorphin, which functions to reduce the effect of dopamine on D1-receptor-bearing striatal projection neurons.

Aberrant neuronal plasticity in the dopamine-depleted striatum

Following dopamine depletion of the striatum, D1-receptor-bearing projecting striatal neurons in the direct pathway display a supersensitive response to D1-receptor-agonist treatment. This supersensitivity develops rapidly following degeneration of the nigrostriatal dopamine terminals (Fig. 3). Thus, three days after unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway, treatment with a D1-receptor agonist results in a robust induction of IEG expression in the striatum. This compares with the absence of a response to the same doses of D1-receptor-agonist treatment in the intact striatum. Dopamine-receptor agonists induce a pronounced contralateral rotation after unilateral 6-OHDA lesions in the rat[39]. This behavioral response was initially thought to result from increased levels of dopamine receptors to compensate for the decreased levels of dopamine. However, alterations in the levels of dopamine receptors are similar to changes in other markers in striatal neurons. Thus, in response to dopamine depletion there is an increase in the number of D2 receptors in indirect striatal neurons and a decrease in the levels of D1 receptors in direct projecting striatal neurons. Treatment with a D1-receptor agonist results in the expression of IEGs in D1-receptor-bearing striatal neurons, despite the decrease in receptor levels. Moreover, reversal of the increased levels of D2 receptors in D2-receptor-bearing striatal neurons does not affect the D1-receptor supersensitivity to treatment with a D1-receptor agonist (C.R. Gerfen, unpublished observations).

The D1-receptor supersensitivity indicated by expression of IEGs in response to D1-receptor agonists differs from cocaine D1-receptor-dependent induction of expression of IEGs in the normal striatum (Fig. 4), in that repeated treatments with a D1-receptor agonist do not result in a decreased response[40]. In fact, repeated treatment with a D1-receptor agonist in animals with unilateral lesions of the nigrostriatal dopamine system results in an enhanced IEG response that has been termed 'priming'. The adaptive mechanism to repeated doses of a D1-receptor agonist in the normal striatum, which involve dynorphin, do not appear to be functional in the dopamine-depleted striatum[31]. Repeated D1-receptor-agonist treatment results in markedly elevated dynorphin levels that are greater than those observed with repeated

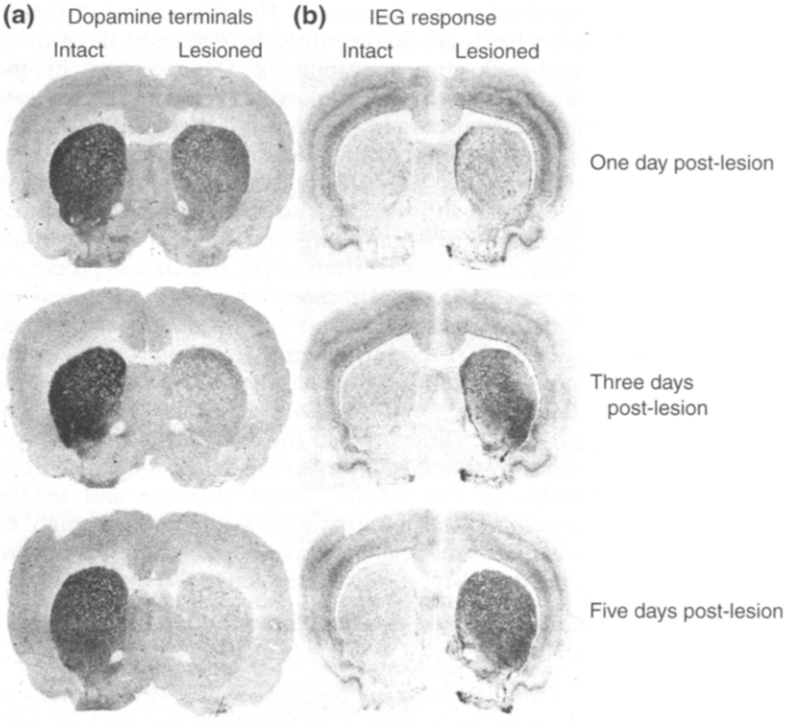

Fig. 3. ***The timecourse of the development of D1-receptor supersensitivity to D1-receptor-agonist treatment following lesion of the nigrostriatal dopamine pathway.*** *In* **(a)** *coronal sections through the striatum are labeled with [³H]mazindol, which binds to presynaptic dopaminergic terminals in the striatum. In* **(b)** *adjacent sections through the striatum display the mRNA encoding the immediate–early gene (IEG)* Arc *with* in situ *hybridization histochemistry. Animals were treated intraperitoneally with the D1-receptor agonist SKF38393 (5 mg/kg) one, three and five days after unilateral lesion of the right nigrostriatal dopamine pathway with 6-hydroxydopamine. At one day post-lesion (top), degeneration of striatal dopamine terminals begins and there is minimal IEG expression in the lesioned striatum. At three days post-lesion (middle), induction of IEG expression by the D1-receptor agonist is apparent in striatal regions in which there is extensive dopaminergic terminal degeneration. By five days post-lesion (bottom), IEG expression induced by the D1-receptor agonist is robust throughout the striatum as dopaminergic-neuron degeneration is complete. Note that there is no significant D1-receptor-agonist-mediated induction of IEG expression in the intact striatum, indicating that the response in the lesioned striatum is supersensitive.*

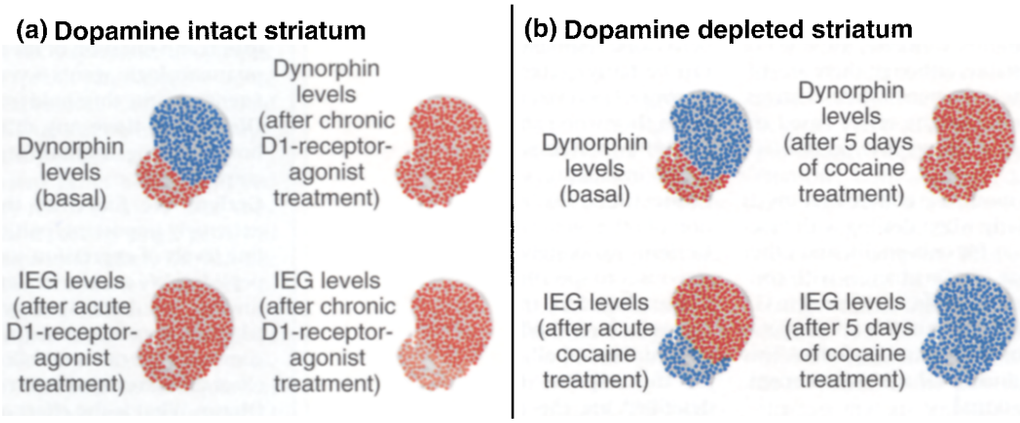

Fig. 4. ***D1-receptor-mediated neuronal plasticity differs in the normal and dopamine-depleted striatum.*** *In the normal striatum* **(a)**, *acute intraperitoneal cocaine treatment (20 mg/kg) results in D1-mediated expression of immediate–early genes (IEGs) in the dorsal striatal region that is complementary to the basal level of dynorphin. Repeated daily treatment with cocaine (five days) results in an increase in dynorphin levels in the dorsal striatum, which functions to decrease the IEG expression response. In the dopamine-depleted striatum* **(b)** *the acute response to intraperitoneal administration of a D1-receptor agonist (SKF38393, 2 mg/kg) results in the induction of IEG expression throughout the striatum. Repeated daily treatment with D1-receptor agonists (14 days) results in an increase in dynorphin expression in the dorsal striatum. However, unlike in the normal intact striatum, in the dopamine-depleted striatum, repeated D1-receptor-agonist activation results in persistent IEG expression in the dorsal striatum. Red indicates high gene expression and blue indicates low gene expression.*

administration of cocaine. However, elevated dynorphin levels are accompanied by decreased IEG expression following activation of D1 receptors in the dopamine-intact striatum, whereas there is no decrease in IEG expression in the dopamine-depleted striatum[40].

There are other differences in the D1 supersensitive response in the dopamine-depleted striatum when compared with the intact striatum. For example, it has been reported that expression of IEGs in the intact striatum by co-activation of D1 and D2 receptors[41], or in response to a D1-receptor agonist is dependent on NMDA-receptor activation[42]. In the dopamine-depleted striatum, D1-receptor agonist treatment results in the expression of IEGs that is independent of D2-receptor stimulation[19], and NMDA- or AMPA-receptor activation[43,44]. Differences in the response of D1-receptor-bearing striatal neurons to D1-receptor-agonist treatments in the normal intact striatum and in the dopamine-depleted striatum suggest that there might be a switch in the signal-transduction mechanisms responsible for D1-receptor supersensitive IEG expression.

Corticostriatal-mediated neural plasticity in the striatum

Stimulation of the corticostriatal pathway results in the expression of IEGs in striatal neurons through activation of glutamate receptors. In a series of studies, it has been shown that electrical stimulation of the cerebral cortex results in IEG expression in striatal neurons that is dependent on activation of the mitogen-activated protein kinase (MAPK) signaling pathway[45,46]. Signal transduction via MAPK is evolutionarily conserved and plays a crucial role in cell growth, being activated by various growth factors that bind tyrosine kinase receptors and lead to the Ras activation of MAPK. Recent studies have shown that Ras activation of MAPK might also be stimulated by Ca^{2+} influx through NMDA receptors[47]. This has led to the suggestion that MAPK is crucially involved in the mechanisms that underlie neuronal plasticity, such as long-term potentiation[48–50].

Interestingly, it has also been reported that, in the striatum, one of the MAPK pathways is activated by glutamate but not by D1-receptor stimulation[51]. D1-receptor-mediated induction of IEG expression has been shown to involve protein kinase A (PKA) and subsequent phosphorylation of CREB (Ref. 52). Both PKA- and MAPK-mediated signal-transduction mechanisms lead to activation of many common IEGs, including *Fos*, through phosphorylation of the transcription factor CREB. However, the MAPK signaling pathway also activates transcription factors that bind the activator protein 1 (AP-1)/TPA response element (TRE) promoter sites of IEGs and results in the induction of distinct IEGs, such as *c-jun* (Ref. 48). Interestingly, D1-receptor stimulation, with either D1-receptor agonists[51] or cocaine[53] has been reported not to induce the expression of the IEG *c-jun*. However, in a survey of IEGs induced by D1-receptor agonists in the dopamine-depleted striatum, it was found that *c-jun* is in fact induced[25]. This suggests that D1-receptor supersensitivity results from a switch in the D1-receptor-mediated signal-transduction mechanisms in the dopamine-depleted striatum.

Concluding remarks

Gene-regulation studies demonstrate that dopamine exerts opposing effects on direct and indirect striatal

Box 1. Discussion

Sealfon: How consistent are the gene changes you have described?
Gerfen: We generally run these experiments with five animals per group and we get fairly consistent results. There is some variability with the cortical stimulation experiments because there are so many variables involved such as location and duration of the stimulus. However, the effect of pharmacologic agents is very stable, although there are differences in the threshold for the induction of these changes.
Olanow: Is there any difference in the effect based on how the drug is administered, whether it is pulsatile or continuous?
Gerfen: We find that the mode of administration is extremely important, particularly when dealing with baseline levels of expression such as for enkephalin and other peptides. We are able to reverse certain changes with continuous vs pulsatile therapy. For example, the G protein G_S, which is normally thought of in terms of the D1 response goes up in D2 neurons following dopamine depletion. This effect can be reversed with continuous D2 agonist treatment.
Obeso: What is the effect of L-dopa?
Gerfen: We did experiments several years ago with Engber and Chase. When we gave L-dopa intermittently, there were changes in D1 neuron peptides that were similar to those seen with D1 agonists, but there was no change in enkephalin.
Bedard: I'm surprised that you couldn't reverse the supersensitivity of the D1 receptor system. We find that continuous L-dopa or dopamine agonist therapy reverses the behavioral responses to a D1 agonist in both the rat and monkey. It is surprising that the c-fos response isn't the same.
Gerfen: It is an interesting point. The signal transduction pathways that are responsible for the generation of this set of immediate-early genes (IEGs) can be dissociated from the physiology of those neurons. Dr Olanow asked if the IEG response was stable following dopaminergic therapies and the answer is 'Yes'. What is highly variable is the behavioral response. If rats show rotational responses, we can be fairly certain that they're going to show an IEG response. However, if they don't show rotation, that doesn't mean they won't show an IEG response. So it's likely that the behavioral response *per se* is not a direct consequence of IEG induction.
Chase: Could the behavioral response relate to the induction of other genes?
Gerfen: Absolutely. But, equating specific behavioral responses to specific gene changes is difficult to establish.
Chase: What is the molecular mechanism of the D1 supersensitivity and what maintains these changes?
Gerfen: First of all, the IEG changes are not maintained, it's the response that is maintained. What we have described are the acute gene responses. We have also looked at the signal transduction pathway. One sees increases in levels of G_S, a protein that's coupled to D1 receptors. So it may be that D1 supersensitivity is related to more efficient coupling to cyclase. On the other hand, it turns out that the elevation of G_S may occur in D2 and not D1 bearing neurons, and here we're able to reverse that elevation in D2 neurons, and have no functional effect. So we don't think it's G_S. We've studied G_{olf}, and this too has no effect. We've looked at the 11 adenylate

projection neurons through the respective segregation of the D1- and D2-receptor subtypes on these neurons. Following degeneration of the nigrostriatal dopamine system, gene expression in the direct and indirect striatal projection neurons is altered in opposite directions. Reversal of these alterations can be affected by selective D1- and D2-receptor-agonist treatment. Striatal dopamine depletion also leads to the development of a supersensitive response to D1-receptor agonists in direct striatal projection neurons that is not reversed with repeated dopamine-agonist treatment. The persistence of dopamine-depletion-induced D1-receptor supersensitivity suggests that there is a switch in the signal-transduction mechanisms of D1-receptor-mediated neuronal plasticity in direct striatal projection neurons. In the intact striatum, D1-receptor stimulation results in adaptive changes that limit neuronal plasticity. However, in the dopamine-depleted striatum, D1-receptor stimulation induces mechanisms resulting in persistent neuronal plasticity. Although some functions of the striatum following dopamine depletion might be restored by dopamine-receptor-agonist treatment, the change in the type of neuronal plasticity in direct striatal neurons persists. This alteration could contribute to or even underlie the motor complications that develop following dopamine-receptor-agonist therapies in PD. Thus, the development of an effective therapy for PD that avoids motor complications could depend on reversing the aberrant form of D1-receptor-mediated neuronal plasticity that develops following degeneration of the nigrostriatal dopamine system.

For further discussion on this topic see Box 1.

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Box 1. Discussion (cont'd)

cyclases, and there is no change in any of these. We've studied protein kinases A and C and there are changes, but they don't appear to be essential. It could be that behavioral changes are related to alterations in the phosphorylation state or in the formation of new gap junctions. At this point, we haven't been able to demonstrate the basis of why this supersensitive response persists.

Walters: Have you tried studying D1 knockouts?

Gerfen: What does one give to D1 knockouts to get a D1 response? We have looked for changes in the signaling pathways in D1 knockouts to see if there are changes downstream in the signaling pathways, but we have no positive findings as yet. However, D3 knockouts do display D1 supersensitivity.

Nutt: What are the implications of your findings for the treatment of Parkinson's disease (PD)?

Gerfen: Well, one consideration is to use D1 agonists from as early as possible in the course of the disease to prevent D1 supersensitivity, as this may relate to the development of L-dopa-induced motor complications.

Smith: Do you find any differences in striatal IEG induction depending on which cortical areas are stimulated?

Gerfen: Yes great differences. It's much easier to elicit IEGs in neocortical areas such as the somatosensory and motor areas than it is from other areas.

Obeso: Has there been any attempt to try to correlate a single change in gene expression with any change in pattern of electrophysiologic activity?

Gerfen: There is evidence in LTP models that changes in gene expression, phosphorylation of cAMP responsive enhancer binding protein (CREB), and changes in the phosphorylation states of various transcription factors are involved in long-term changes in physiologic activity. How these gene changes equate to changes in physiologic activity is not known. *Arc* is a very likely candidate to be involved in plasticity because it is expressed in dendrites. There is nice work relating changes in *Arc* expression to synaptic input. The real question is what specific physiologic response is altered by a change in gene expression. Candidates could be genes involved in the cytoskeletal scaffolding that couple physiologic responses to signaling pathways.

Olanow: With so many different genes being expressed and regulated simultaneously, it's very likely that they will interact with each other, and that no single gene will necessarily correlate with a behavioral effect. Do you not think this is likely to be some complex network of changes rather that any one single gene?

Gerfen: Yes, we are gearing up to study multiple genes with the microarray approach and hope that this will allow us to establish the pattern of up- and down-regulation changes that are important for behavioral change. But, I agree that this is likely to be very complicated and not necessarily an easy task to solve.

Smith: Have you noticed any gene change in striatal interneurons?

Gerfen: Yes, they do show changes. In addition, there are changes in GABAergic interneurons that are involved in plasticity. They don't display LTP for the most part, but they do display LTD – and that's thought to be related to the type of cAMP kinase that they're expressing.

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