Modulation of Neuronal Differentiation by Dopamine Receptors

Fazal Arain

Brain development is a prolonged process that requires a tight regulation of spatiotemporal events. Neuropsychiatric disorders, like schizophrenia and bipolar disorder have been hypothesized to have a developmental etiology despite the fact that the manifestations of the symptoms of these diseases occur later in life. It has been suggested that developmental perturbation of neuronal architecture in specific regions of the forebrain is responsible for the manifestations of these diseases. Dopamine is a neurotransmitter that appears prior to synaptogenesis and expression of the dopamine system is particularly high in the regions implicated in neuropsychiatric disorders. The receptors for dopamine are classified into two sub-families which trigger unique intracellular signaling cascades and modulation of the physiology of these receptors appear to result in permanent alteration in the morphology of dopaminoceptive neurons. A number of approaches, that include modulation of activity of these receptors by different chemical compounds, altered receptor trafficking and developmental deletion of dopamine receptors, have complemented these conclusions. It appears that modulation of neuronal morphology depends upon the type of dopamine receptor activated, the distinct intracellular signaling cascade triggered and the region of the brain that express these neurons. The cellular mechanisms involved in the modulation of neuronal morphology are still under investigation. Alterations in the intracellular calcium ion concentration upon activation of dopamine receptors could lead to the triggering of down stream signals linked to the modulation of neuronal cytoskeleton and hence neuronal morphology. Studies conducted on receptor-specific modulation of neuronal differentiation could help us better understand the pathophysiology of neuropsychiatric disorders.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: fazal.m.arain.1@vanderbilt.edu.

Brain development is a prolonged and dynamic process that begins in the prenatal period and continues through adolescence[1, 2]. This phenomenon can be appreciated by looking at the examples of spatiotemporal dynamics of dendritic differentiation and synaptogenesis[3]. Therefore these dynamics need to be considered when studying brain development.

Both genetic and environmental factors have profound effects on the formation and integrity of brain architecture. Abnormalities in the development of the brain are linked to neurological and psychiatric disorders, despite the fact that these diseases manifest their symptoms later on in life[4]. It is now believed that abnormalities in the brain architecture occur much earlier then the actual appearance of the symptoms of these diseases[5]. Therefore it can be postulated that the manifestations of these diseases could be the result of convergence of many different developmental pathological processes linked to these diseases[6].

As the brain develops the roles played by neurotransmitters change significantly[7]. In the adult nervous system, neurotransmitters are primarily involved in the transmission of information across synapses[8]. On the one hand, they perform a much more complicated role of modulating the connectivity and subsequent function of different regions of the brain during development[8]. Therefore the perturbation of the biology of neurotransmitters during development could be associated with alterations in the brain architecture and consequently with neurological and psychiatric diseases.

Two areas of the brain, namely medial prefrontal cortex (MFC) and striatum (STR) have been linked to complex brain functions such as cognition[9], motivation and planning etc. These two areas have also been implicated in neuropsychiatric disorders[6, 10, 11]. Therefore the neurotransmitter systems expressed during and after development of MFC and STR, could play a crucial role in the development of these neuropsychiatric disorders.

DOPAMINE: THE NEUROTRANSMITTER

Dopamine is a neurotransmitter that is synthesized in dopaminergic neurons from the amino acid tyrosine via a series of reactions, the rate limiting step of which is mediated by the enzyme tyrosine hydroxylase. The principle dopaminergic fiber systems in the brain include the nigrostriatal (that connects the substantia nigra pars compacta to the dorsal striatum), mesolimbic pathway (that connects ventral tegmental area to the ventral striatum) and the mesocortical pathway (that connects ventral tegmental area to the prefrontal cortex). Receptors for dopamine

are expressed in particularly high concentration in two regions of the brain; namely the MFC and STR. The role of dopamine in these areas has been linked to cognition, reward, learning and movement[12].

Dopamine can exert an excitatory or an inhibitory influence on its target cell by binding to the appropriate receptor subtypes. The action of dopamine is terminated by reuptake into the presynaptic neuron by the action of dopamine transporter; following which it can be either repackaged into vesicles or degraded by the enzymes monoamine oxidase and catechol-O-methyltransferase. Dopamine binds to five distinct receptors called dopamine receptor 1, 2, 3, 4 and 5 (D1-5). These are transmembrane receptors that are coupled to G protein, and hence they belong to G protein coupled receptors (GPCR) family. Based on intracellular signaling mechanisms, subtypes of the coupled G proteins and sequence of homology, dopamine receptors have been classified into two subfamilies; namely the D1-like receptor subfamily (that includes D1 and D5) and D2-like receptor subfamily (that includes D2, D3 and D4)[13]. D1 receptor expressing neurons co-express the neuropeptides dynorphin and substance P, while the D2 receptor expressing neurons co-express enkephlins[14, 15]. The D1-like receptors couple to $G_{\alpha s/olf}$ proteins and the D2-like receptors couple to $G_{\alpha o/i}$ protein. The activation of D1 and D2-like receptor subfamilies trigger intracellular signaling cascades that eventually result in accumulation and depletion of intracellular cAMP respectively. It is reported that there also exists another category of dopamine receptors, namely the D1 and D2 receptor heterodimers, that are coupled to $G_{\alpha q/11}$ protein and are linked to the phospholipase C pathway[16]. The signaling transduction through the $G_{\alpha q/11}$ has been shown to result in the release of calcium ions (Ca^{2+}) from intracellular stores[17].

It is traditionally believed that the D1 and D2 receptors are expressed by distinct neurons. Studies using insitu-hybridization techniques have shown that majority of D1 and D2 receptors are expressed on distinct neurons[18]. Segregation of neurons that express D1 and D2 receptor RNA has also been shown in striatum[18]. On the other hand evidence has been presented negating this view and introducing the idea that D1 and D2 receptor are also co-expressed on the same neurons (most probably as heterodimers). The evidence for physical interaction includes co-immunoprecipitation, western blotting and immunocytochemistry[17, 19]. Evidence has also been presented by studies that used in situ hybridization[20], single cell RT-PCR[18, 21] and electrophysiology[19] to support this model.

Studies have been conducted to define the functional characteristics of D1-D2 heterodimer expressing cells. Using HEK 293T and COS7 cells transfected with D1 and D2 cDNA, it has been shown that co-stimulation via D1 receptor agonist (SKF 81297) and D2 receptor agonist (quinpirole) can result in the stimulation of phospholipase C pathway and an increase in accumulation of intracellular Ca^{2+} ions[17]. Treatment of D1-D2 transfected HEK cells with phospholipase C inhibitor, U71322, was shown to be sufficient to deplete cytosolic Ca^{2+} ions[22]. In fact, it was shown that this Calcium signaling pathway could be blocked at different steps of the pathway, for example by $G_{q/11}$ inhibitor (YM254890), by antagonist of intracellular inositol triphosphate receptors (2-aminoethoxydiphenyl borate) and by depletion of intracellular calcium stores (by thoxydiphenyl borate)[22]. A calcium signal was also generated upon administration of dopamine receptor agonist SKF 83959 (that specifically stimulates the phospholipase C pathway), but it was not as strong as that generated by the co-administration of SKF 83959 and quinpirole. Interestingly, the administration of either SCH23390 (D1 receptor antagonist) or raclopride (D2 receptor antagonist) was sufficient to eliminate the signaling cascades triggered by co-administration of SKF 83959 and quinpirole[22]. These data support the hypothesis that D1/D2 receptor heterodimers are a distinct entity with unique activity profile.

As mentioned earlier, the dopamine system has been shown to be associated with a number of crucial physiological activities. Alterations in the dopamine system have also been implicated in neurological and psychiatric disorders including Huntington's disease, Parkinson's Disease, schizophrenia and bipolar disorder[6, 10, 11]. In fact, drugs used in the treatment of some these disorders target the dopamine system[23, 24]. It must also be appreciated that due to the complexities of these disorders, no single experimental model can address all aspects of these diseases.

TEMPORO-SPATIAL EXPRESSION OF DOPAMINE RECEPTORS

Dopamine is one of the neurotransmitters that appear very early in the developing brain[25]. In fact the expression of dopamine begins prior to synaptogenesis[26]. Dopaminergic neurons are born around the time of telencephalic vesicle formation, from neuroepithelial cells (the cell population from which precursor brain cells are derived)[26]. It has been observed that tyrosine hydroxylase expression (a marker for dopaminergic neurons) is detectable by embryonic day 11 (E11) in mice, E12-13 in rats, E14 in rabbits and E30 in monkeys[27, 28]. Although the expression of dopamine is evident in the developing striatum and cortex, its expression is also seen in close proximity of the neuroepithelial cells[28]. This unique spatiotemporal expression of dopamine

advocates its role as a modulator of neurogenesis. Although numerous studies have provided replicable evidence for the spatiotemporal expression of dopaminergic neurons in different species, the precise estimation of the dynamics of dopamine receptor expression has been a difficult task[29]. Unreliability of antibodies and the nonspecificity of chemical compounds that serve as dopamine receptor agonists and antagonists have contributed to this scenario[29]. Therefore a lot of studies have used alterative techniques such as *in situ* hybridization and RT PCR to define the expression pattern of dopamine receptors[30, 31]. Lately, using laser capture microdissection, it has been shown that the transcripts of dopamine receptors can be detected as early as E12 in mouse brain[29], which coincides with the birth of striatal neurons[32]. It was shown that the most abundant transcript of dopamine receptor during development was of D2[29]. It was also seen that while D1, D2 and D5 receptors show a progressive increase in expression with increasing age, D3 and D4 receptors have an oscillatory expression profile[29].

Following their birth, the dopaminergic neurons extend axonal branches that follow a dorsal trajectory towards the midbrain and then grow ventro-rostrally towards their targets in telencephalon, traversing the diencephalon[33]. At around E17, in rats, dopaminergic axon bundles start to enter the developing striatum and some of them continue to proceed towards the cortex and eventually reach their appropriate targets[33]. It is also worth noting that the trajectory of growing axons and dendrites is influenced by environmental cues (molecules that bind to their appropriate receptors on growth cones; an area present at the tips of axons and dendrites). These environmental cues include ephrins, netrin, semaphorins and slits[33]. In a recent study, the opposing roles of netrin (that binds to DCC/ deleted in colorectal cancer) and slits (that binds to robo) in guiding dopaminergic tract was described[34]. Using a zebrafish model it was shown that the absence of robo2 resulted in deviation of dopaminergic axons towards midline and that this defect was partially restored upon silencing netrin expression with morpholinos in these mutants[34]. These opposite roles of netrin and slit signaling are consistent with *in vitro* models[35]. DCC knockout mouse model has been relatively uninformative in this regard as the homozygous mutants die soon after birth but the heterozygous mice do show an altered dopamine expression pattern in the brain and an abnormal blunt response to amphetamine treatment[36].

Interestingly, modulation of D1 and D2 receptor expressed on GABAergic neurons can alter the migration of these neurons[37]. It was shown that D1 receptor activation caused an increase while D2 receptor activation caused a decrease in neuronal migration[37]. These results were also complemented with findings seen with tissue obtained from D1 and D2 receptor knock out mice[37]. In light of these findings it can be postulated that disruption of the dopamine system during a defined embryonic period can have significant and long lasting effects on neuronal architecture[38] that have functional implications[39].

In the adult brain, dopamine system orchestrates complex spatiotemporal sequence of neural events that allows information from cortex to flow to the basal ganglia and hence modulate complex functions such as motor control, cognition and behavior. The circuitry involved in the motoric function is well defined[40]. In short, the motor and pre-motor cortical areas send excitatory glutamatergic neuronal fibers that synapse predominantly with the medium spiny neurons of the striatum. The medium spiny neurons of striatum also receive dopaminergic input from substantia nigra pars compacta. Of these striatal medium spiny neurons, the ones that co-express substance P and D1 receptors send GABAergic fibers to globus pallidus par interna and hence become a part of the *direct pathway*. On the other hand medium spiny neurons that co-express D2 receptors and enkephlins send GABAergic fibers to globus pallidus par externa and hence become a part of the *indirect pathway*. The direct pathway eventually allows disinhibition of its thalamic and cortical targets while indirect pathway results in their inhibition. It is becoming increasingly evident that these pathways are not only involved in motoric control but also in the control of cognitive functions[40].

EVIDENCE OF DOPAMINE SYSTEM'S INVOLVEMENT IN NEURONAL DEVELOPMENT

As mentioned earlier, in light of its temporal expression pattern, dopamine appears to be a very strong candidate in modulating neuronal differentiation. In fact, there are several lines of evidence that link the dopamine system to neuronal differentiation (**Figure 1**).

1: Stimulation of D1 and D2 receptors has distinct consequences on neuronal morphology: A number of studies designed to investigate the effects of modulating the activity of D1 and D2 receptors on neuronal morphology have been conducted in an *in vitro* model. It has been seen that the application of D1 receptor agonist, SKF 38393, causes a reduction in the extent of neurite outgrowth of neurons derived from MFC[41, 42] while it causes an increase the extent of neurite outgrowth in striatal neurons[43]. Interestingly, these effects can be attenuated by the addition of SCH23390 (a D1 receptor blocker)[41, 43]. On the contrary, addition of quinpirole (a D2 receptor agonist) results in an increase in neurite outgrowth[44] and branching[45] of neurons derived from MFC.

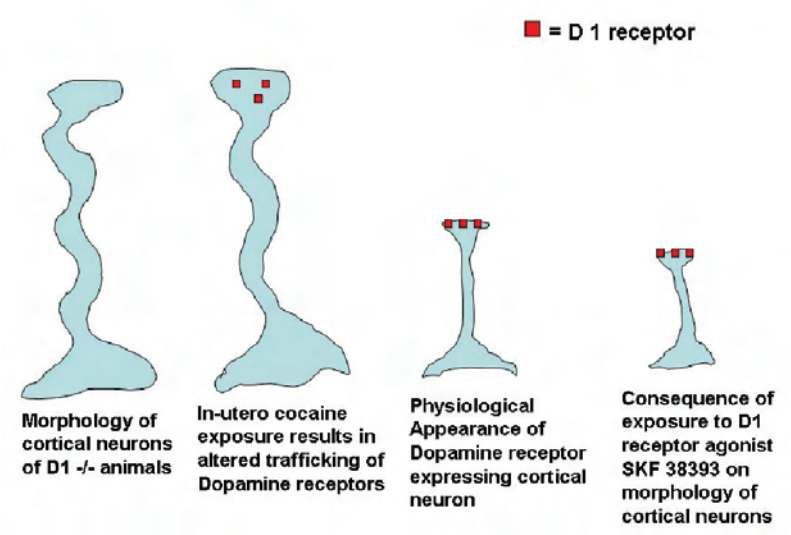

Figure 1. | **Modulation of D1 receptor causes alteration in neuronal morphology.**

Studies conducted on neuronal cell lines transfected with D2-like receptor cDNA also show an increase in neurite length and branching upon exposure to quipirole[46]. The activity of adenyl cyclase, as modulated by D1 and D2-like receptors, has been linked to these changes in neuronal morphology.

D1 receptor agonist SKF83959 that signals through the $G_{\alpha q/11}$ pathway, activates the phospholipase C pathway[16]. Although there is a controversy regarding the precise receptor population whose stimulation results in activation of $G_{\alpha q/11}$ signaling cascade[16, 47], it has been seen that exposure to SKF 83959 results in a robust increase in neurite outgrowth and a marked change in the morphology of neurons derived from MFC (Arain & Stanwood, unpublished observations). Therefore it appears that the neuronal morphology of dopaminoceptive neurons is not just modulated by the type of dopamine receptor stimulated but also by the specific intracellular signaling cascade triggered.

2: Absence of D1 receptor results in increased neurite length: Evidence for the role of dopamine receptor in modulating neuronal differentiation has also been presented using *in vivo* models. As can be predicted from the *in vitro* model (described above) which shows that since the stimulation of D1 receptor causes a decrease in neurite outgrowth, deletion of D1 receptor should have an opposite impact. This hypothesis was supported by studies done in the D1 receptor null mouse model that showed that the dendritic morphology of MFC neurons, was long and tortuous[48]. These finding were not present in the visual or parietal cortices (areas receiving little dopaminergic innervation)[48].

3: Cocaine exposure modulates dopamine receptor trafficking and neuronal morphology: In utero exposure to cocaine is associated with cognitive deficit in children[2]. Cocaine is also an inhibitor of catecholamine transporters[49]. It has been shown that exposure to cocaine during a defined developmental window causes permanent changes in the morphology of dopaminoceptive neurons[42]. These changes include elongation of dendrites and a characteristic "wavy" morphology. These changes are also accompanied by altered D1 receptor trafficking and uncoupling of D1 receptors to G protein[42, 50]. It is suggested that inhibition of dopamine transporter (by cocaine) on the presynaptic membrane results in increased concentration of dopamine in the synaptic space and this scenario is compensated by reduced surface expression of D1 receptors and their uncoupling to $G_{\alpha s}$ protein on the postsynaptic membrane. This model complements the findings seen in D1 receptor null mouse[48]. In both of these experimental models it is seen that the unavailability of functional D1 receptors result in an increase neurite outgrowth and a wavy morphology of MFC neurons. Therefore these findings are consistent with the hypothesis that inactivation (via mal-trafficking or deletion) or activation of D1 receptors has distinct impacts on the neuronal morphology.

MODULATION OF MICROTUBULE-ASSOCIATED PROTEIN-2 (MAP2) PHOSPHORYLATION RESULTS IN ALTERATION OF NEURITE MORPHOLOGY

As mentioned earlier, the stimulation of D1 receptors leads to intracellular accumulation of cAMP which leads to the activation of protein kinase A (PKA)[23]. Microtubule associated protein2 (MAP2) promotes the assembly and stabilization of microtubules[51] (that holds the cytoskeletal structure intact). MAP2 activity is modulated by phosphorylation at a number of its sites by PKA[52] and dephosphorylation by protein-phosphatase 2A (PP2A)[53]. It has been shown that one of the mechanisms involved in altering neurite length in wildtype primary neuronal cultures, following D1 receptor agonist exposure, involves the phosphorylation of amino acid residues in MAP2 by the action of PKA[54]. These findings are consistent with the observations seen in the *in vivo* model of D1 over-expressing transgenic mice[54]. Interestingly, a MAP2 deficient mouse model shows a decrease in microtubule density and a reduction in dendritic length of hippocampal neurons[55]; thus providing yet another evidence for a crucial role of MAP2 in neuronal differentiation.

ROLE OF DOWNSTREAM EFFECTORS OF Ca^{2+} SIGNALS IN NEURONAL MORPHOLOGY

As mentioned earlier, the modulation of dopamine receptors results in alteration of the Ca^{2+} signals. Therefore it is likely that Ca^{2+} signals modulates down stream signaling cascades that eventually results in altering neuronal morphology[56]. One of these down stream signals is the modulation of calcium/calmodulin-dependent protein kinase II (CamKII) activity[57]. It's been shown that constitutive activity of CamKII results in inhibition of dendritic growth, while its inhibition causes an increase in dendritic growth[57]. Furthermore, in another study it was shown that CamKII signaling involves the a number of signaling molecules that activate MAP kinase which goes on to phosphorylate the transcription factor CREB and hence eventually stimulate the transcription of Wnt-2 which directly stimulate dendritic growth[58]. Hence Ca^{2+} signaling seems to be the key architect of neuronal morphology.

CONCLUSION

The temporo-spatial pattern of dopamine system makes it an interesting candidate to study the mechanisms of brain development. The perturbation of dopamine system results in permanent alterations in neuronal morphology, the evidence for which is provided by both *in vitro* and *in vivo* models. This perturbation appears to be modulated by a number of factors that include the expression pattern of dopamine receptor subtypes, the brain region expressing these receptors and the intracellular signaling cascades triggered by them. Alteration of neuronal morphology appears to include both the extent of neurite outgrowth and their branching pattern, which could eventually translate into alteration of neuronal circuitry. Since the changes in dopamine system appear to cause alteration in the brain architecture of regions rich in dopaminoceptive neurons (regions also implicated in the pathophysiology of neuropsychiatric disorders), the dopamine system becomes an obvious candidate to study the developmental etiology of neuropsychiatric disorders. Further studies that target both the expression pattern and the pharmacology of these receptors need to be conducted to define the effects of modulation of dopamine receptors on neuronal morphology.

REFERENCES

1. Levitt P (2003). Structural and functional maturation of the developing primate brain. *J Pediatr.* **143**: S35-45.
2. Stanwood GD & Levitt P (2004). Drug exposure early in life: functional repercussions of changing neuropharmacology during sensitive periods of brain development. *Current Opinion in Pharmacology.* **4**: 65-71.
3. McGlashan TH and Hoffman RE (2000). Schizophrenia as a disorder of developmentally reduced synaptic connectivity. *Arch Gen Psychiatry.* **57**: 637-48.
4. Sun D, Stuart GW, Jenkinson M, Wood SJ, McGorry PD, Velakoulis D, van Erp TG, Thompson PM, Toga AW, Smith DJ, Cannon TD and Pantelis C (2008). Brain surface contraction mapped in first-episode schizophrenia: a longitudinal magnetic resonance imaging study. *Mol Psychiatry.* **14** (10): 976-986.
5. Weinberger DR (1995). From neuropathology to neurodevelopment. *Lancet* **346**: 552-557.
6. Lewis DA and Levitt P (2002). Schizophrenia as a disorder of neurodevelopment. *Annu Rev Neurosci.* **25**: 409-32.
7. Buznikov GA, Shmukler YuB and Lauder JM (1999). Changes in the physiological roles of neurotransmitters during individual development. *Neurosci Behav Physiol.* **29**: 11-21.
8. Rice D and Barone S (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect.* **108** Suppl 3: 511-533.
9. Koechlin E, Ody C and Kouneiher F (2003). The architecture of cognitive control in the human prefrontal cortex. *Science.* **302**: 1181-1185.
10. Rubia K, Smith AB, Brammer MJ, Toone B and Taylor E (2005). Abnormal brain activation during inhibition and error detection in medication-naive adolescents with ADHD. *Am J Psychiatry.* **162**: 1067-1075.
11. Drevets WC, Ongur D and Price JL (1998). Neuroimaging abnormalities in the subgenual prefrontal cortex: implications for the pathophysiology of familial mood disorders. *Mol Psychiatry.* **3**: 220-226, 190-191.
12. Stanwood GD (2008). Protein-protein interactions and dopamine D2 receptor signaling: a calcium connection. *Mol Pharmacol.* **74**: 317-319.
13. Sibley DR and Monsma FJ (1992). Molecular biology of dopamine receptors. *Trends in Pharmacological Sciences.* **13**: 61-68.
14. Greengard P, Allen PB and Nairn AC (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron.* **23**: 435-447.
15. Ingham CA, Hood SH, Mijnster MJ, Baldock RA and Arbuthnott GW (1997). Plasticity of striatopallidal terminals following unilateral lesion of the dopaminergic nigrostriatal pathway: a morphological study. *Exp Brain Res.* **116**: 39-49.
16. Rashid AJ, O'Dowd BF, Verma V and George SR (2007). Neuronal Gq/11-coupled dopamine receptors: an uncharted role for dopamine. *Trends Pharmacol Sci.* **28**: 551-5.
17. Lee SP, So CH, Rashid AJ, Varghese G, Cheng R, Lança AJ, O'Dowd BF and George SR (2004). Dopamine D1 and D2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal. *J Biol Chem.* **279**: 35671-8.
18. Le Moine C and Bloch B (1995). D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. *J Comp Neurol.* **355**: 418-426.
19. Aizman O, Brismar H, Uhlén P, Zettergren E, Levey AI, Forssberg H, Greengard P and Aperia A (2000). Anatomical and physiological evidence for D1 and D2

dopamine receptor colocalization in neostriatal neurons. *Nat Neurosci.* **3**: 226-30.

20. Le Moine C & Gaspar P (1998). Subpopulations of cortical GABAergic interneurons differ by their expression of D1 and D2 dopamine receptor subtypes. *Brain Res Mol Brain Res.* **58**: 231-236.
21. Surmeier DJ, Eberwine J, Wilson CJ, Cao Y, Stefani A and Kitai ST (1992). Dopamine receptor subtypes colocalize in rat striatonigral neurons. *Proc Natl Acad Sci U S A.* **89**: 10178-10182.
22. Rashid AJ, So CH, Kong MM, Furtak T, El-Ghundi M, Cheng R, O'Dowd BF and George SR (2007). D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proc Natl Acad Sci U S A.* **104**: 654-659.
    This article was chosen because it provides evidence for the existence of functional D1/D2 heterodimers that are associated with Gαq/11 protein. Using both in vitro and in vivo models they show that these receptors possess unique pharmacology. They go on to show that stimulation of these receptors results in triggering signaling cascades that cause activation of CamKII.
23. Missale C, Nash SR, Robinson SW, Jaber M and Caron MG (1998). Dopamine receptors: from structure to function. *Physiological Reviews.* **78**: 189-225.
24. Strange PG (2008). Antipsychotic drug action: antagonism, inverse agonism or partial agonism. *Trends Pharmacol Sci.* **29**: 314-21.
25. Puelles L and Verney C (1998). Early neuromeric distribution of tyrosine-hydroxylase-immunoreactive neurons in human embryos. *J Comp Neurol.* **394**: 283-308.
26. Levitt P, Harvey JA, Friedman E, Simansky K and Murphy EH (1997). New evidence for neurotransmitter influences on brain development. *Trends in Neurosciences.* **20**: 269-274.
27. Levitt P and Rakic P (1982). The time of genesis, embryonic origin and differentiation of the brain stem monoamine neurons in the rhesus monkey. *Brain Research.* **256**: 35-57.
28. Bhide PG (2009). Dopamine, cocaine and the development of cerebral cortical cytoarchitecture: a review of current concepts. *Semin Cell Dev Biol.* **20**: 395-402.
29. Araki KY, Sims JR and Bhide PG (2007). Dopamine receptor mRNA and protein expression in the mouse corpus striatum and cerebral cortex during pre- and postnatal development. *Brain Res.* **1156**: 31-45.
    This paper provides a detailed analysis of spatiotemporal expression of all the five dopamine receptors by quantifying their mRNA expression. The technique used, laser capture microdissection, to collect tissue samples from specific brain areas appear to be a reliable approach for the purpose of these experiments. The estimated mRNA levels also appeared to co-relate with the receptor protein expression patterns.
30. Lidow MS and Rakic P (1992). Scheduling of monoaminergic neurotransmitter receptor expression in the primate neocortex during postnatal development. *Cereb Cortex*. **2**: 401-16.
31. Jung AB and Bennett JP (1996). Development of striatal dopaminergic function. I. Pre- and postnatal development of mRNAs and binding sites for striatal D1 (D1a) and D2 (D2a) receptors. *Brain Res Dev Brain Res*. **94**: 109-20.
32. Ohtani N, Goto T, Waeber C and Bhide PG (2003). Dopamine modulates cell cycle in the lateral ganglionic eminence. *J Neurosci*. **23**: 2840-2850.
33. Van den Heuvel DM and Pasterkamp RJ (2008). Getting connected in the dopamine system. *Prog Neurobiol*. **85**: 75-93.
34. Kastenhuber E, Kern U, Bonkowsky JL, Chien CB, Driever W and Schweitzer J (2009). Netrin-DCC, Robo-Slit, and heparan sulfate proteoglycans coordinate lateral positioning of longitudinal dopaminergic diencephalospinal axons. *J Neurosci*. **29**: 8914-26.
35. Lin L, Rao Y and Isacson O (2005). Netrin-1 and slit-2 regulate and direct neurite growth of ventral midbrain dopaminergic neurons. *Mol Cell Neurosci*. **28**: 547-555.
36. Flores C, Manitt C, Rodaros D, Thompson KM, Rajabi H, Luk KC, Tritsch NX, Sadikot AF, Stewart J, Kennedy TE (2005). Netrin receptor deficient mice exhibit functional reorganization of dopaminergic systems and do not sensitize to amphetamine. *Mol Psychiatry*. **10**: 606-612.
37. Crandall JE, McCarthy DM, Araki KY, Sims JR, Ren JQ, Bhide PG (2007). Dopamine receptor activation modulates GABA neuron migration from the basal forebrain to the cerebral cortex. *J Neurosci*. **27**: 3813-22.
38. Stanwood GD, Washington RA, Shumsky JS and Levitt P (2001). Prenatal cocaine exposure produces consistent developmental alterations in dopamine-rich regions of the cerebral cortex. *Neuroscience*. **106**: 5-14.
39. Stanwood GD and Levitt P (2003). Repeated i.v. cocaine exposure produces long-lasting behavioral sensitization in pregnant adults, but behavioral tolerance in their offspring. *Neuroscience*. **122**: 579-83.
40. Lewis SJ and Barker RA (2009). Understanding the dopaminergic deficits in Parkinson's disease: insights into disease heterogeneity. *J Clin Neurosci*. **16**: 620-625.
41. Reinoso BS, Undie AS and Levitt P (1996). Dopamine receptors mediate differential morphological effects on cerebral cortical neurons in vitro. *Journal of Neuroscience Research*. **43**: 439-453.
42. Stanwood GD and Levitt P (2007). Prenatal exposure to cocaine produces unique developmental and long-term adaptive changes in dopamine D1 receptor activity and subcellular distribution. *J Neurosci*. **27**: 152-157.
    This article was chosen because it provides evidence for the modulation of neuronal morphology due to altered dopamine receptor trafficking and uncoupling to G protein as a consequence of prenatal cocaine exposure. They also examine the effects of SKF 38393 (a D1 receptor agonist) on neuronal morphology and provide yet another evidence of how altering the physiology of dopamine receptors can result in permanent alterations in neuronal morphology.
43. Schmidt U, Beyer C, Oestreicher AB, Reisert I, Schilling K and Pilgrim C (1996). Activation of dopaminergic D1 receptors promotes morphogenesis of developing striatal neurons. *Neuroscience*. **74**:

453-460.
44. Reinoso BS, Undie AS and Levitt P (1996). Dopamine receptors mediate differential morphological effects on cerebral cortical neurons in vitro. *J Neurosci Res*. **43**: 439-453.
45. Todd RD (1992). Neural development is regulated by classical neurotransmitters: dopamine D2 receptor stimulation enhances neurite outgrowth. *Biol Psychiatry*. **31**: 794-807.
46. Swarzenski BC, Tang L, Oh YJ, O'Malley KL and Todd RD (1994). Morphogenic potentials of D2, D3, and D4 dopamine receptors revealed in transfected neuronal cell lines. *Proc Natl Acad Sci U S A*. **91**: 649-653.
47. Dai R, Ali MK, Lezcano N and Bergson C (2008). A crucial role for cAMP and protein kinase A in D1 dopamine receptor regulated intracellular calcium transients. *Neurosignals*. **16**: 112-123.
48. Stanwood GD, Parlaman JP and Levitt P (2005). Anatomical abnormalities in dopaminoceptive regions of the cerebral cortex of dopamine D(1) receptor mutant mice. *J Comp Neurol*. **487**: 270-282.
49. Ritz MC, Cone EJ and Kuhar MJ (1990). Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure-activity study. *Life Sci*. **46**: 635-645.
50. Jones LB, Stanwood GD, Reinoso BS, Washington RA, Wang HY, Friedman E and Levitt P (2000). In utero cocaine-induced dysfunction of dopamine D1 receptor signaling and abnormal differentiation of cerebral cortical neurons. *J Neurosci*. **20**: 4606-4614.
51. Sanchez C, Diaz-Nido J and Avila J (2000). Phosphorylation of microtubule-associated protein 2 (MAP2) and its relevance for the regulation of the neuronal cytoskeleton function. *Prog Neurobiol*. **61**: 133-168.
52. Goldenring JR, Vallano ML and DeLorenzo RJ (1985). Phosphorylation of microtubule-associated protein 2 at distinct sites by calmodulin-dependent and cyclic-AMP-dependent kinases. *J Neurochem*. **45**: 900-905.
53. Gong CX, Wegiel J, Lidsky T, Zuck L, Avila J, Wisniewski HM, Grundke-Iqbal I and Iqbal K (2000). Regulation of phosphorylation of neuronal microtubule-associated proteins MAP1b and MAP2 by protein phosphatase-2A and -2B in rat brain. *Brain Res*. **853**: 299-309.
54. Song ZM, Undie AS, Koh PO, Fang YY, Zhang L, Dracheva S, Sealfon SC and Lidow MS (2002). D1 dopamine receptor regulation of microtubule-associated protein-2 phosphorylation in developing cerebral cortical neurons. *J Neurosci*. **22**: 6092-6105. **This paper describes the modulation of the phosphorylation state of MAP2 following in vitro exposure to D1 receptor agonists and how it translates into altered neuronal morphology. The authors also report studies conducted on a D1 receptor overexpressing mouse model, that exhibit decreases in apical dendritic length of cortical pyramidal neurons and increases in phosphorylation state of MAP2 protein.**
55. Harada A, Teng J, Takei Y, Oguchi K and Hirokawa N (2002). MAP2 is required for dendrite elongation, PKA anchoring in dendrites, and proper PKA signal transduction. *J Cell Biol*. **158**: 541-549.
56. Zheng JQ and Poo MM (2007). Calcium signaling in neuronal motility. *Annu Rev Cell Dev Biol*. **23**: 375-404.
57. McAllister AK (2000). Cellular and molecular mechanisms of dendrite growth. *Cereb Cortex*. **10**: 963-973.
58. Wayman GA, Impey S, Marks D, Saneyoshi T, Grant WF, Derkach V and Soderling TR (2006). Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. *Neuron*. **50**: 897-909.

FURTHER INFORMATION

Gregg Stanwood's Lab:
http://www.mc.vanderbilt.edu/root/vumc.php?site=stanwoodlab