

Dopamine: the rewarding years

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Dopamine has moved from being an insignificant intermediary in the formation of noradrenaline in 1957 to its present-day position as a major neurotransmitter in the brain. This neurotransmitter is involved in the control of movement and Parkinson's disease, the neurobiology and symptoms of schizophrenia and attention deficit hyperactivity disorder. It is also considered an essential element in the brain reward system and in the action of many drugs of abuse. This evolution reflects the ability of several famous names in neuropharmacology, neurology and psychiatry to apply new techniques to ask and answer the right questions. There is now excellent knowledge about the metabolism of dopamine, dopamine receptor systems and the structural organisation of dopamine pathways in the brain. Less is known about the function of the different receptors and how the various dopamine pathways are organised to produce normal behaviour, which exhibits disruption in the disease states mentioned. In particular, we have very limited information as to why and how the dopamine system dies or becomes abnormal in Parkinson's disease or a neurodevelopmental disorder such as schizophrenia. Dopamine neurones account for less than 1% of the total neuronal population of the brain, but have a profound effect on function. The future challenge is to understand how dopamine is involved in the integration of information to produce a relevant response rather than to study dopamine in isolation from other transmission systems. This integrated approach should lead to greater understanding and improved treatment of diseases involving dopamine.

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Abbreviations: ADHD, attention deficit hyperactivity disorder; CNS, central nervous system; COMT, catechol-*o*-methyltransferase; CREB, c-AMP response element-binding protein; DAT, dopamine membrane transporter; ECD, electrochemical detection; fMRI, functional magnetic resonance imaging; GABA, γ -aminobutyric acid; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine; IUPHAR, International Union of Pharmacology; L-DOPA, L-dihydroxyphenylalanine; MAO, monoamine oxidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRI, magnetic resonance imaging; NIMH, National Institute for Mental Health; NMDA, *N*-methyl-D-aspartate; 6-OHDA, 6-hydroxydopamine; PET, positron emission tomography; phMRI, pharmacological magnetic resonance imaging; SERT, serotonin membrane transporter; SPET, single-photon emission tomography; VMAT, vesicular monoamine transporter; VTA, ventral tegmental area

Introduction

This is an age in which celebrity status appears to be a human aim. Dopamine has certainly achieved that status in the neurotransmitter world with a highly successful book (Awakenings by Oliver Sachs), which inspired a play by Harold Pinter and was then converted to an equally compelling film in 1990 starring Robin Williams and Robert De Niro. The book and the film brought to the written word and the silver screen the dramatic effects of the use of L-DOPA in patients. Dopamine has also made frequent appearances in television science programmes so bringing the transmitter to the attention of a wider public. The celebrity status of dopamine, in contrast to many present-day 'celebrities', is however one of substance.

Understanding of the importance of dopamine for normal brain function has had a major impact on the treatment of important neurological (such as Parkinson's disease) and psychiatric disorders (such as schizophrenia and, more

recently, attention deficit hyperactivity disorders (ADHD)). Dopamine also has a social importance as the neurotransmitter that plays a central role in the mechanism of action of the major substances of abuse and, in particular, those associated with dependence and addiction. The impact of dopamine extends beyond treatment of disease to the development of experimental and diagnostic tools with which we have been able to investigate brain neurotransmitter function in the living human brain and offer important future diagnostic opportunities. The early positron emission tomography (PET) studies on neurotransmission exploited the availability of suitable dopaminergic ligands to show dopamine loss in Parkinson's disease in the living patient.

These advances have been achieved by an impressive cast of scientists from around the world, making the best use of the methodology available at the time to provide vital information that has gradually filled in at least some of the missing pieces of the dopamine jigsaw. Both Arvid Carlsson with Paul Greengard and Eric Kandel (2000), and Julius Axelrod, with Ulf von Euler and Sir Bernard Katz (1970), were awarded

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Nobel Prizes. Arvid Carlsson for his work on dopamine systems and Julius Axelrod for his work on catecholamine metabolism including the identification of the importance of *O*-methylation (Axelrod, 1957). Many other famous names in pharmacology have been involved in the story and it is fitting in the present context of the 75th Anniversary of the BPS that it was Henry Dale who first examined the biological activity of dopamine in the same year (1910) as George Barger and James Hill who synthesised 3,4-dihydroxyphenylethylamine at The Wellcome Laboratories in London, and indeed it was Henry Dale who first suggested the shorter name dopamine in 1952 (Hornykiewicz, 1986).

The aim of this short review is to highlight some of the important steps that have taken place in the evolution of our understanding of the function of dopamine with specific reference to its role in the brain. This does not attempt to be a balanced account as there is an obvious bias to the event this special issue celebrates – 75 years of the BPS and British Pharmacology. The major examples that I draw on are the involvement of dopamine in Parkinson's disease and the development of symptomatic treatment for this disease. Another example is the importance of dopamine in the treatment of schizophrenia and emerging ideas about the neurodevelopmental aspects of mental disorder with a shift in interest from dopamine in the accumbens to understanding the complex interaction between dopamine function in cortical areas, the prefrontal cortex in particular and subcortical dopaminergic systems. The final area is the role of dopamine in reward and the mechanism of action of drugs of abuse. The emphasis on brain function purely reflects the research interests of the author and it is important to stress that dopamine is not restricted to the brain, but has important functional implications outside the brain including cardiovascular function.

A brief history of dopamine

While dopamine was first synthesised in 1910, it was not until the mid-1950s that it began to emerge as a substance of importance in its own right rather than just an intermediary in the formation of noradrenaline. There are excellent reviews of the early history of dopamine by Oleh Hornykiewicz (1986; 2002), who himself played such an important role in the initial studies showing loss of dopamine in post-mortem brains from Parkinsonian patients and the subsequent use of L-DOPA in such patients (Birkmayer & Hornykiewicz, 1961). The present account will be restricted to the key steps in the story.

Herman Blaschko at Oxford, who worked on monoamine oxidase (MAO) in the early years (see also Youdim & Bakhle, this issue), was one of the first to suggest that dopamine may be physiologically significant, a view substantiated by studies showing that dopamine could lower blood pressure and this effect was potentiated by inhibition of MAO (Blaschko, 1957). Incidentally, my first *viva voce* examination was conducted by Herman Blaschko and in such a manner that it was a factor that led me to stay in research; he also asked the first question after my first presentation at a BPS meeting. At about the same time, Kathleen Montagu (1957) and Arvid Carlsson, together with Lindqvist, Magnusson and Waldeck (Carlsson *et al.*, 1958), identified dopamine in the brain, and in 1959, the Swedish group showed that dopamine was found in the

striatum in high concentrations where there was very little noradrenaline (Bertler & Rosengren, 1959). These findings led to the subsequent work by Hornykiewicz on post-mortem Parkinsonian human brain (Ehringer & Hornykiewicz, 1960).

The next important step was the development of the fluorescence histochemical method by Hillarp & Falk for the visualisation of catecholamine and indoleamine (5-HT) neurones and their pathways in the brain (for details of the methodology, see Corrodi & Johnsson, 1967). With this method, Kjell Fuxe and Annika Dahlstrom together with others, including a young Urban Ungerstedt who later played an essential role in the development of the microdialysis technique, produced the first evidence for neuronal pathways containing dopamine that projected from the substantia nigra to the striatum and the ventral tegmental area (VTA) to the limbic areas of the rat brain (Dahlström & Fuxe, 1965; Andén *et al.*, 1966). This relatively simple plan of the dopamine pathways in the brain has been refined over the years with dopaminergic input to the frontal cortex being identified (see later), but the basic plan remains. It was at this point that I entered research and my PhD used the fluorescence histo-

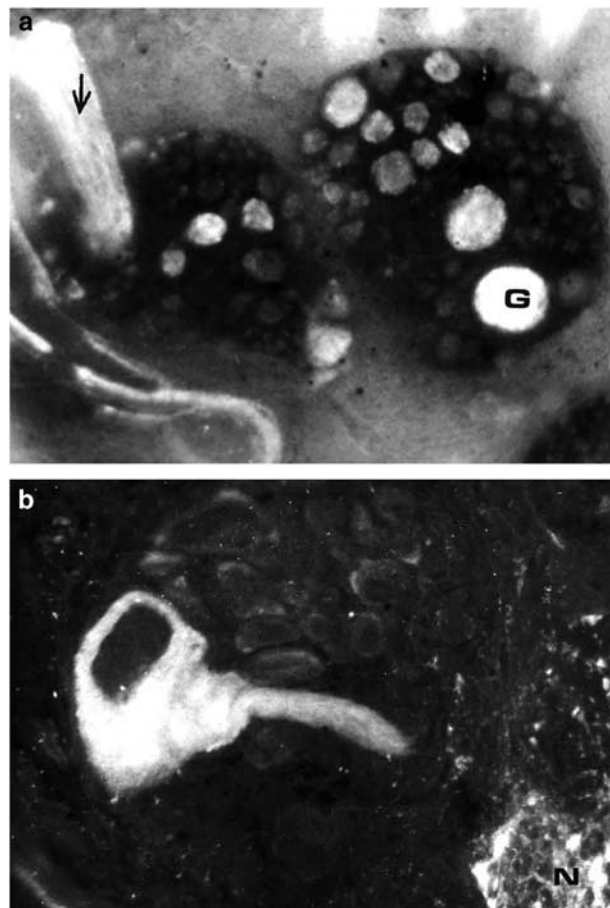


Figure 1 Giant dopamine-containing neurone in the left pedal ganglia of *Planorbis corneus*. The upper photograph is a whole mount of the two ganglia showing the giant dopamine neurone (G, as dopamine produces a green fluorescence with the Falk-Hillarp method) in the left ganglion. The other fluorescing cells are yellow and contain 5-HT (serotonin). The lower photograph is a section through the left pedal ganglion showing the large dopamine neurone with its axon. This neurone was used in subsequent metabolic and electrophysiological studies (Berry & Cottrell, 1973).

chemical technique to visualise dopamine and 5-HT in the ganglia ('brains') of snails and the leech. My lucky break came with the identification of a giant neurone in the pond snail *Planorbis corneus* containing dopamine (Marsden & Kerkut, 1970; Figure 1). Up to that time, the only dopamine cells identified in invertebrates were very small and therefore unsuitable for electrophysiological investigation, which was the essential technique for pharmacological structure–activity studies at that time, as exemplified by work carried out by Walker and Woodruff using the snail *Helix aspersa* (Walker *et al.*, 1968).

Although by the mid-1960s the presence of dopamine in the brain and its loss in Parkinson's disease had been established, the effectiveness of L-DOPA treatment demonstrated and dopamine pathways in the brain visualised, there was still debate about whether dopamine had a neurotransmitter role related to its presence in neurones or some other function. Marthe Vogt described a "constant discharge of dopamine from terminals of nigrostriatal neurones" (Portig & Vogt, 1969), but still expressed doubts that dopamine was a conventional neurotransmitter (Vogt, 1973). Annika Dahlström (1973) was equally cautious when she stated that in the central nervous system (CNS) "the central neurones synthesise store and, in all likelihood, release noradrenaline, dopamine and 5-hydroxytryptamine". It was the identification of specific receptors and the development of selective agonists and antagonists that finally removed the caution.

Dopamine receptors, pathways and their function

While the 1960s and 1970s were mainly concerned with establishing an independent role for dopamine and making the first tentative steps in determining its physiological and behavioural functions, the 1980s were the receptor years with

the development of techniques allowing study of links between specific receptors and the function of specific dopamine pathways within an increasingly complex system of neuronal interactions.

The first evidence for the presence of a dopamine receptor in brain came from experiments showing the stimulation of adenylyl cyclase activity by dopamine (Kebabian *et al.*, 1972). A little later Philip Seeman began to search for the brain's antipsychotic receptor and identified a site that could be labelled by both dopamine and haloperidol and the binding of both was inhibited by nanomolar concentrations of unlabelled haloperidol. This site was originally called the 'neuroleptic/dopamine receptor' (Seeman *et al.*, 1976), but was later renamed the dopamine D₂ receptor in an important paper that classified the dopamine receptors into D₁ and D₂ based on their pharmacology and coupling to adenylyl cyclase (Kebabian & Calne, 1979). This receptor remains a key target for antipsychotic drugs, although it is also known to be linked to various adverse side effects associated with such drugs (see later). It was not until the early 1980s that the dopamine D₂ receptor in the pituitary, thus the receptor involved in the control of prolactin release, was shown to inhibit adenylyl cyclase, in contrast to the stimulation produced by the dopamine D₁ receptor.

The 1980s also saw the arrival of molecular biology and gene cloning of receptors, which led to further dopamine receptors being identified, although they all fitted neatly into the original D₁/D₂ classification based on sequence homology, adenylyl cyclase response and pharmacology (Table 1). Several members of the BPS were involved in this phase of dopamine receptor identification including Geoff Woodruff (Woodruff *et al.*, 1977) and Philip Strange (Strange, 1991). While discovery of receptor binding sites was important, the next step was to determine their distribution in the brain using autoradiography and then relating that distribution to what we knew about dopamine pathways and their function. More recently, attention has moved to understanding the intra-

Table 1 General overview of the properties of the dopamine receptor subtypes

	'D ₁ -like'		'D ₂ -like'		
	D ₁	D ₅	D _{2(short)/(long)}	D ₃	D ₄
Amino acids	446(h, r)	477(h) 475(r)	414/443(h) 415/444(r)	400(h) 446(r)	387(h, r)
Antagonists	SCH 23390	?	Spiperone Raclopride Clozapine S33084	Spiperone Raclopride Clozapine L745870	Spiperone Raclopride Clozapine S33084
Homology					
With D ₁ receptor	100	82	44	44	42
With D _{2(short)}	44	49	100	76	54
Receptor localization	Caudate/putamen, nucleus accumbens, olfactory tubercle, hypothalamus, thalamus, frontal cortex	Hippocampus, thalamus, lateral mamillary nucleus, striatum, cerebral cortex (all low)	Caudate/putamen, nucleus accumbens, olfactory tubercle, cerebral cortex (low)	Nucleus accumbens, olfactory tubercle, islands of Calleja, cerebral cortex (low)	Frontal cortex, midbrain, amygdala, hippocampus, hypothalamus, medulla (all low), retina
Response	Adenylyl cyclase ↑	Adenylyl cyclase ↑	Adenylyl cyclase ↓	Adenylyl cyclase ↓	Adenylyl cyclase ↓

This classification is based on the IUPHAR Compendium of Receptor Characterization and Classification. Complete details are available in Alexander *et al.* (2004). Differences between the human and rat forms of the receptors are indicated by (h) and (r), respectively. The D₂ receptor exists in short and long variant spliced forms; these forms have similar, but not identical, pharmacology. This table has been adapted from one originally produced by Philip Strange.



Figure 2 David Marsden (centre) with Peter Jenner on his right at a meeting in 1984 (photograph kindly provided by Peter Jenner).

cellular proteins and pathways that are regulated by different dopamine receptor pathways. We now know that receptors do not act in isolation, but exist in a complex that includes regulatory and scaffolding molecules, which in turn could offer novel drug targets, but more importantly may hold the key to understanding how environmental factors produce long-term or irreversible effects on neurotransmission.

As already mentioned, the original fluorescence histochemical studies, derived from approximately 15,000–20,000 mesencephalic neurones, had identified the major dopamine-mediated pathways. These can be summarised as follows:

1. The nigrostriatal pathway from the substantia nigra to the caudate putamen (dorsal striatum) is the pathway primarily associated with Parkinson's disease. Both D_1 (excitatory) and D_2 (inhibitory) receptors are found in the striatum, where the D_2 receptor is located both pre- and postsynaptic, while the D_1 receptors are postsynaptic. The important role of these two receptors in the dorsal striatum is to modulate the function of the direct and indirect pathways, from the motor cortex and back to the cortex *via* the thalamus, involved in the control and initiation of motor plans. D_2 receptors in the striatum also act as the presynaptic inhibitory autoreceptor and antagonism of these receptors by the classical antipsychotic drugs, such as haloperidol, results in increased dopamine release, which may be an important factor in the development of tardive dyskinesias by these drugs, while antagonism of striatal postsynaptic D_2 receptors can explain the initial extrapyramidal side effects of those drugs (Marsden *et al.*, 1975). This is an appropriate point to introduce David Marsden (Figure 2) – no direct relation to me – who had such an impact on British and international neurology and neuropharmacology through his work on Parkinson's disease and antipsychotic drugs before his sudden early death in 1998. His long-time collaborator at Kings College, Peter Jenner, however, has ensured continuation of the work on Parkinson's disease (see later).
2. The pathways from the VTA to the limbic areas of the brain (accumbens, ventral striatum and amygdala), known as the mesolimbic pathway, and the mesocortical pathway from the VTA to the cortex (medial, prefrontal, cingulate

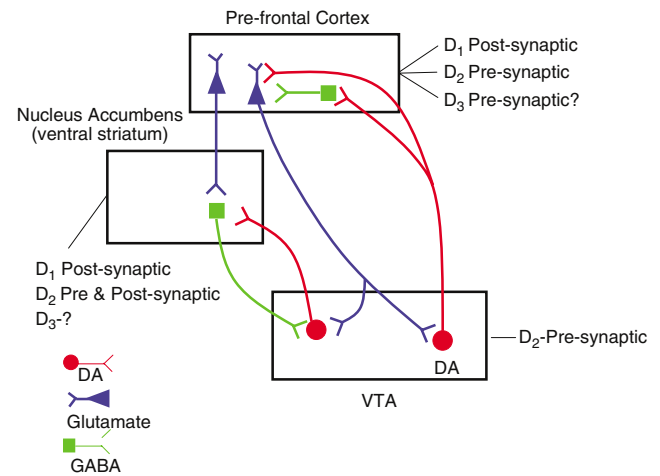


Figure 3 Simplified diagram showing the neural pathways linking the prefrontal cortex, the nucleus accumbens and the ventral tegmental area (VTA). At least three transmitters are involved in these links – dopamine, glutamate and GABA. The positive symptoms of schizophrenia can be reduced by antagonism of postsynaptic D_2 receptors in the accumbens, while the negative and cognitive symptoms are associated with reduced dopamine function in the prefrontal cortex. Agonists at the D_1 receptors can increase glutamate function and D_2/D_3 antagonism may also increase glutamate function by inhibiting GABA. The numbers of D_2 receptors in the prefrontal cortex are low; here the D_3 receptor may have a functionally similar role to the D_2 receptor.

- and entorhinal cortex) are both associated with reward and schizophrenia, although, as will be discussed later, our understanding of the role of dopamine in schizophrenia has evolved over the past 30 years (Figure 3) and we cannot ignore a role for the dorsal striatum in aspects of addiction. The D_1 receptor has the widest distribution of all the dopamine receptors in the brain and it is found in association with the mesolimbic pathway as is the D_2 receptor. Importantly, the dopamine D_3 receptor, which has certain functional and pharmacological similarities to D_2 receptors, is specifically localised in limbic areas and is not found in the striatum, so is an obvious potential drug target. There are also relatively more D_4 receptors in the limbic dopaminergic regions than in the striatum, although overall expression of D_4 receptors is low, with the relative abundance of dopamine receptors in the rat CNS being $D1 > D2 > D3 > D5 > D4$ (Jaber *et al.*, 1996).
3. The final major dopamine pathway runs from the arcuate and periventricular nuclei of the hypothalamus to the intermediate lobe of the pituitary and the median eminence. This system forms the tuberoinfundibular dopamine pathway important in the inhibitory control of prolactin and is mediated by the D_2 receptor. Increased prolactin release is a side effect of conventional antipsychotic drugs and changes in plasma prolactin were very effectively used by Tim Crow and colleagues to show that D_2 receptor antagonism was the key action in the antipsychotic effect of these drugs (Johnstone *et al.*, 1978). Furthermore, one of the first clinical uses of a selective dopamine agonist (bromocriptine) was to treat infertility due to hyperprolactinaemia. In the past few years, the functional distinction between dorsal and ventral striatum has become blurred through the work of several research groups including that of Trevor Robbins

in Cambridge. The idea that psychostimulants act only on reward and reinforcement mechanisms located in the ventral (accumbens) striatum is an oversimplification, as some of these effects can be mediated by sites in the dorsal striatum. The most important overlap between the areas occurs with cognitive function and behavioural correlations appear better served by thinking in terms of a dorsolateral to ventromedial functional organisation (see review by Voorn *et al.*, 2004).

From receptors and pathways to function: methodological advances

Early studies attempting to identify the functions of dopamine in the brain were limited by the experimental tools available. The first pharmacological tool was reserpine, but it was not selective for dopamine as it depleted all amines from their storage vesicles. In a similar manner, the tyrosine hydroxylase inhibitor, α -methyl-para-tyrosine, prevented the synthesis of both dopamine and noradrenaline. Amphetamine was little better as it released both of the catecholamines, although later studies demonstrated that dopamine was the major contributor to the gross behavioural effects observed such as hyperlocomotion and stereotypy. Following the discovery of dopamine receptors and the link with both schizophrenia and Parkinson's disease, more selective compounds began to appear that acted as agonists or antagonists at specific dopamine receptor subtypes and this opened up the field of dopamine behavioural pharmacology to which Susan Iversen and her laboratory in Cambridge made a very important contribution.

Apart from the emerging drugs, an even older compound had a profound influence on dopamine research. 6-Hydroxydopamine (6-OHDA) had been shown to deplete peripheral noradrenaline in a paper written by Laverty in the *British Journal of Pharmacology* in 1965. Unable to cross the blood-brain barrier, it was not until 6-OHDA was given by central injection that a similar depletion was seen in the brain, which also included depletion of dopamine (Laverty & Taylor, 1970; Uretsky & Iversen, 1970). Subsequently, Urban Ungerstedt in Sweden used local injections into specific dopamine pathways and the well-known rotational model was born, and it was now possible to produce specific lesions of dopamine pathways, independent of effects on noradrenergic pathways. My first *in vivo* studies on the rat in 1971 used the Ungerstedt rotational model to look at dopamine-serotonin interactions. Over the next few years, numerous papers appeared that linked dopamine to various behaviours including locomotor activity, stereotyped behaviours, learning, motivation, food intake, reward and intracranial self-stimulation.

Another neurotoxic agent has also played an important role in our understanding of dopamine in Parkinson's disease by producing a more reliable model of the disease than that produced by 6-OHDA. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was originally synthesised in the 1960s, but it came to wider notice in the 1980s when it was tracked down by Langston at Stanford University as the cause of a sudden increase in Parkinsonian-like symptoms in young adults in Northern California. The drug was identified as a contaminant in some locally produced 'synthetic heroin', self-administered by the individuals showing the Parkinsonian-like symptoms.

We now know that it is not MPTP that cause the dopamine neurotoxicity, but that MPTP is metabolised by MAO-B to MPP⁺, either within dopamine neurones or astrocytes, and it is the MPP⁺ that causes neurodegeneration by inhibiting mitochondrial complex 2 and the production of free radicals. Peter Jenner and many others (see also Youdim & Bakhle, this issue) have used the MPTP model in various animal species to identify potential new drug targets not just for the symptomatic treatment of Parkinson's disease but also to test strategies that might lead to more permanent recovery (Treseder *et al.*, 2000). The use of L-DOPA, in the form of carbidopa, has been a very successful approach to the pharmacological replacement of the deficient dopamine system in the disease, but there are problems such as the diminishing response with time and development of the 'on-off' profile of the treatment. Furthermore, certain cognitive and emotional side effects occur and, in some patients, a drive to abuse the drug develops. Some of these problems can be overcome by the use of selective dopamine agonist drugs such as ropinirole and this approach may in future be further developed using drugs with more selectivity for a particular subtype or the use of partial dopamine agonists. Other potential drug targets are other neurotransmitter systems that modulate dopamine function, such as adenosine. The use of non-pharmacological approaches including neurosurgery and deep brain stimulation also have a role in symptomatic treatment. The long-term aim, however, is the development of treatments that either prevent loss of dopamine in the first place or replace the lost dopamine neurones. In the latter situation, transplantation of embryonic mesencephalic neurones has been tried, but with limited success, probably due to poor survival, and a future alternative will be the use of stem cells engineered to produce dopamine. With all these approaches, initial studies using the MPTP model will be an essential step.

Successful use of 6-OHDA and MPTP is dependent upon the ability to show you have produced an adequate lesion of either the dopamine or noradrenaline system and this requires reliable sensitive assay methods for both. The early methods involved tissue extraction and then, in some cases, chromatographic (paper or column) separation, followed finally by fluorimetric measurement. The assays were laborious and lacked the sensitivity for measurements from small brain regions. These problems were overcome by the arrival of HPLC separation combined with electrochemical detection (ECD). The ECD method was developed by Ralph Adams, with whom I was lucky enough to work during 1977 in Lawrence, Kansas, and he had the vision to see that the detector could be miniaturised and implanted into the brain so that constant recordings could be made. The aim was to develop a method for on-line measurement of dopamine and 5-HT release or, at least, the extracellular levels of these amines (Conti *et al.*, 1978). Ralph and I demonstrated the method of *in vivo* voltammetry at the summer meeting of the BPS held at Nottingham in 1978, my last whole animal demonstration at a BPS meeting. We then presented the data at the European Neuroscience Association meeting in Florence where we met Urban Ungerstedt who was developing the microdialysis method for the same purpose. To cut a long story short, the microdialysis method combined with HPLC and ECD has provided a reliable method for detecting changes in extracellular dopamine (Zetterström *et al.*, 1983) both in response to drugs and behavioural situations but the response time is

slow, as normally the minimum collection time is 5 min or more. Voltammetry on the other hand allows real-time measurement, but there are issues with the interpretation of the signal obtained as the electrodes available are not specific to dopamine but also detect the metabolites of dopamine. Both approaches, however, have made a considerable impact on our understanding of the relationship between dopamine release and behavioural events.

In the last decade or so, other techniques have played an important role in research into the functional role of dopamine neurones in the brain as well as in our understanding of other mediator systems. Central among these methods is the use of genetically engineered mice to investigate functional aspects of specific receptors as well as the membrane dopamine transporter (DAT). We are also starting to see the application of both functional and pharmacological magnetic resonance imaging (fMRI or phMRI) and PET techniques to the investigation of preclinical issues relating to dopamine function. For example, the use of phMRI with the blood oxygen level-dependent (BOLD) response will allow the identification in the living animal of brain regions that are either activated or deactivated by a specific drug. When combined with appropriate animal models, we should be able to get information about how drug responses may differ between the normal and the disease state.

Schizophrenia: from the dopamine hypothesis to neurodevelopmental abnormality

The dopamine hypothesis of schizophrenia grew out of the observation in the 1960s that amphetamine-induced psychosis had similarities with schizophrenia and amphetamine acts by increasing catecholamine function, through dopamine in particular. Finally, Philip Seeman (Seeman *et al.*, 1976) showed that haloperidol and dopamine bind to the same site and then demonstrated an excellent correlation between drug dose required to treat the positive symptoms of schizophrenia and the ability of the drug to bind to the dopamine D₂ receptor. The importance of the D₂ receptor in the action of these drugs was further clarified, by Tim Crow and colleagues, in an elegant clinical study that compared the effects of the two isomeric forms of flupenthixol, only one of which had affinity for the D₂ receptor, on the symptoms of the disease and on the increase in plasma prolactin produced by D₂ antagonism. The results showed that it was only the isomer (α -flupenthixol) with affinity for the D₂ receptor that reduced the symptoms and increased prolactin (Johnstone *et al.*, 1978). The question remained as to whether schizophrenia was associated with an identifiable abnormality in the dopaminergic systems in the brain. Extensive studies on post-mortem brain found no consistent presynaptic changes but suggested that postsynaptic D₂ receptor numbers were increased in the accumbens, although there remains debate about the importance of these findings and, in particular, whether the change is drug induced or a consequence of the disease. More recently, using SPET imaging methodology, Laurelle and co-workers have shown that schizophrenic patients release more dopamine than healthy controls (Laruelle *et al.*, 1996). One interesting and sustained report from Gavin Reynolds demonstrated laterality in the change in dopamine with an increase in the right, but not left, amygdala (Reynolds, 1983).

While evidence accumulated for D₂ receptor antagonism being important in the treatment of the positive symptoms of schizophrenia, these drugs were far from perfect due to their side-effect profile and their failure to treat either the negative or cognitive symptoms of the disease. Antagonism of dopamine receptors in the mesolimbic dopamine pathway was inferred from the reduction of the positive symptoms and similar antagonism of D₂ receptors in the striatum explained the acute Parkinsonian-like side effects. However, the long-term tardive dyskinesias were more difficult to account for and even today are still a bit of an enigma. The concept of presynaptic receptors and autoinhibitory control of transmitter release was originally proposed for peripheral noradrenaline in a publication by Brown and Gillespie in the *Journal of Physiology* in 1957 and then extended by Salomon Langer in the early 1970s, taking into account developments in our understanding of receptor mechanisms. Long-term antagonism of the presynaptic D₂ receptor results in prolonged increase in dopamine release, which may 'break through' the postsynaptic receptor blockade to produce the excessive expression of striatal function (Marsden & Jenner, 1980). The obvious aim of the drug developers was to produce new antipsychotic drugs with fewer extrapyramidal (striatal) side effects. One approach to the problem was to exploit the new information about the distribution of the various dopamine receptor subtypes and the obvious choice was to target the D₄ receptor that was found in the limbic regions but not the striatum; however, this approach yielded no drugs of value. Clozapine, which was shown to have fewer extrapyramidal side effects and a complex pharmacology, formed the blueprint for the next approach and subsequent drugs such as risperidone and olanzapine had reduced D₂ antagonism combined with activity at other sites including 5HT_{2A}, so the 'magic bullet' approach was replaced by drugs with a 'rich pharmacology'. The new atypical antipsychotics have reduced the number of side effects, but not successfully treated the negative symptoms or cognitive impairments and the search is on for appropriate receptor targets.

The growing emphasis on the cognitive deficits and the need to understand the neural system that underlies them has altered the approach to schizophrenia research in recent years. This has been coupled with the emergence of powerful brain imaging techniques that have demonstrated organic changes in the schizophrenic brain – identified before the arrival of MRI, again by Tim Crow, but enhanced in value by MRI. The evidence of nondegenerative ventricular enlargement and reduced hippocampal cortical size supported the concept of schizophrenia being neurodevelopmental in origin. The change in cortical organisation with the disorder has resulted in a re-evaluation of the role of dopamine in schizophrenia. It is now apparent that some of the core symptoms (negative symptoms and cognitive deficits) of schizophrenia are not related to increased dopamine, but rather to decreased dopamine function in the prefrontal cortex (the mesocortical dopamine system). Thus, schizophrenia may result from a primary neurodevelopmental disruption to the cortical brain areas causing reduced prefrontal cortical dopamine function, which in turn, together with reduced glutamate, enhances mesolimbic dopamine function. This means re-evaluation too of the dopamine treatment strategies and the possible value of partial agonist treatment, first suggested by Arvid Carlsson in 1988, to produce increased prefrontal cortex function while decreasing

mesolimbic function. An example of this approach is given by aripiprazole, which has often been described as a dopamine system stabilizer. This compound is a partial agonist at dopamine D₂ receptors and at 5HT_{1A} receptor, but a high-affinity agonist at 5HT_{2A} receptors.

Another approach is to exploit the differential locations of dopamine D₂ and D₃ receptors. The two receptors have very similar pharmacology, but there are very limited numbers of D₂ receptors in the prefrontal cortex while D₃ receptors are relatively abundant. Amisulpride is equally potent as an antagonist at D₂ and D₃ receptors and shows efficacy in treating both positive and negative symptoms with low extrapyramidal side effects (Figure 3). A third approach may be to selectively activate postsynaptic D₁ receptors in the prefrontal cortex either alone or in conjunction with upregulation of the glutamate NMDA receptor.

The problem of cognitive deficits, including poor attention and executive function, remains and there is now an emphasis on developing suitable animal models of this aspect of schizophrenia, combined with modelling the potential early environmental factors. One such model is the postweaning isolation reared rat, extensively worked on in the U.K. by Trevor Robbins and co-workers in Cambridge and ourselves in Nottingham, which shows relevant behavioural deficits and neuropharmacological features as well as neuropathological changes to the cortex and hippocampus. The phencyclidine model based on NMDA receptor function disruption has also undergone detailed further evaluation by Judith Pratt and Brian Morris in Glasgow (Cochran *et al.*, 2003) and shows considerable promise. Current views support the hypothesis that schizophrenia is a complex interaction between environmental and genetic factors and, in recent years, the gene encoding the enzyme catechol-*o*-methyltransferase (COMT), which metabolises catecholamines including dopamine, has attracted most attention. COMT exists in low- and high-activity forms and the high-activity form is more prevalent in patients with schizophrenia. This finding could explain the low dopamine function in the prefrontal cortex as the dopamine nerve terminals in this area do not have dopamine reuptake sites and are thus dependent on enzymatic degradation to inactivate dopamine. Interestingly, other studies by Daniel Weinberger at NIMH have shown alternative mutations, which decrease COMT activity and so increasing dopamine function in the prefrontal cortex and these individuals show enhanced executive functioning. These findings suggest that COMT inhibitors, such as tolcapone, could help restore normal cognitive functioning in schizophrenia.

One final point of interest is the possible similarities in terms of the involvement of prefrontal cortical function in the cognitive problems associated with schizophrenia and ADHD – advance in one may help the other.

Dopamine: dependence, reward and drugs of abuse

There is strong evidence that drugs of abuse which produce reward, cause reinforcement and may cause dependence, also increase dopamine release in mesolimbic regions. These drugs include opiates, cannabis, nicotine as well as drugs acting directly on presynaptic dopamine terminals (amphetamine, methamphetamine and cocaine; see also Corbett *et al.*, this

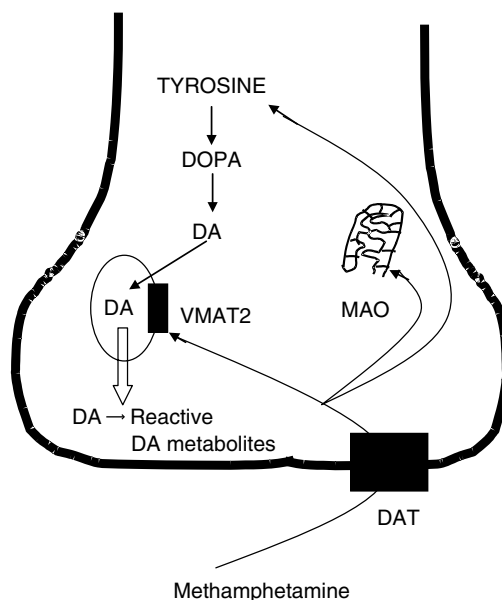


Figure 4 Possible mechanism for the neurotoxicity produced by methamphetamine. This amine enters dopamine neurones/terminals via the dopamine transporter (DAT) and then releases dopamine from vesicles and prevents storage there by blocking the vesicular transporter (VMAT2). Methamphetamine also inhibits the activity of mitochondrial MAO involved in the catabolism of dopamine while increasing its synthesis by stimulating tyrosine hydroxylase. The net result is an increase in cytosolic dopamine, which may be metabolised to reactive dopamine metabolites, resulting in neuronal damage and degeneration. Adapted from Larsen *et al.* (2002).

issue). There is vast literature on this socially and scientifically important area of research. During 2004–2005, the U.K. Foresight Programme, managed by the Office of Science and Technology, ran a project that enquired into how scientific and technological advances might impact on our understanding of addiction and drug use over the next 20 years. The project was led by David Nutt, Trevor Robbins and Gerry Stimson. One outcome of the project was the production of 15 comprehensive reviews of topics relating to drugs of abuse and addiction which are available on the Foresight website (www.foresight.gov.uk).

I will restrict my present comments to a few key items relating to the pharmacology of the drugs that remain unanswered after 40 years of dopamine research.

Monoamine uptake across neuronal cell membranes carried out by the appropriate transporters, such as DAT and SERT, is now well understood and has resulted in the development of numerous important drugs that act on these transporter systems (see also Iversen, this issue). The pioneering work in this area by Leslie Iversen dates back to the 1960s. The importance, however, of the other uptake systems associated with monoamine nerve terminals, which control vesicular monoamine uptake in the brain (VMAT2) is much less understood. Can differences in the effects of structurally related drugs on vesicular uptake help explain differences in their rewarding and reinforcing properties? For example, amphetamine and methamphetamine, but not methylphenidate (Ritalin), block vesicular uptake, which may explain the differences in response between the first two drugs and Ritalin. More information is required about the relationship between these two transporter systems.

There is a rise in methamphetamine use in the U.S.A. and in South-East Asia, particularly among the young, with growing concern about the ability of the drug to cause dopaminergic neurodegeneration. How and under what conditions does such degeneration occur? One possibility is that methamphetamine enters the dopamine neurones using the membrane DAT, and releases dopamine from vesicles while also preventing storage there and increasing synthesis (Figure 4). The net result is raised levels of cytosolic dopamine, which can be converted into reactive dopamine metabolites, as the normal intraneuronal route of catabolism through MAO is inhibited by methamphetamine (Larsen *et al.*, 2002). Ecstasy is another drug of concern, although in that case the concern is about loss of serotonergic innervation. Both are amphetamines and so common basic mechanisms may be involved in their neurotoxic potential.

Stimulant drugs remain the major treatment for ADHD, yet we still know little about possible long-term use of such compounds on the developing brain. Likewise, drug abuse is common in young humans, yet most experimental studies use 'adult' animals. There is a need for more work on how drugs of abuse might affect the developing brain and for us not to consider children as 'little adults' when it comes to drugs and dopamine.

What about the future?

Our understanding of the role of dopamine in the brain continues to unfold and I am sure BPS members will continue to be involved in this process as there is much to do. We know a lot about the dopamine D₁ and D₂ receptors, but less about the other three dopamine receptors, although such information will emerge with the use of knockout mice. For example, use of male and female D₅ knockout mice have shown this receptor

to be involved in the rewarding aspects of sexual behaviour – receptivity in females and intromissions in males. New advances will occur in our knowledge of how dopamine receptors control or modulate different aspects of behaviour.

Another knockout has raised an intriguing question as to whether reward can occur in the absence of dopamine. Claire Cannon and Richard Palmiter have shown that mice unable to synthesise dopamine, as they lack tyrosine hydroxylase, are still able to demonstrate a preference for sucrose, which is a natural reward process.

We can expect great progress to be made in the understanding of the link between dopamine receptor occupation and altered gene expression. There is already evidence that this is effected not just through the classical adenylyl cyclase and CREB phosphorylation pathways, but may involve other quite different routes such as mechanisms dependent on β -arrestin-2. A further alternative for the D₂ receptor is action through prostate apoptosis response-4, a molecule linked to apoptosis and neuronal death.

Finally, the emphasis so far has been on investigating dopamine in isolation from other transmitter systems. The increasing awareness of the neuronal organisation of cortical areas and their links with subcortical regions makes it essential that we investigate the expression of physiological and behavioural responses involving dopamine in an integrated manner. Dopamine neurones account for less than 1% of the neurones in the brain, but changes in the levels or functional state of brain dopamine can have profound behavioural effects, suggesting that the role of dopamine is not to relay specific information but to integrate information relevant to the incoming biologically important stimuli. Dopamine research has yielded great scientific rewards and clinical benefits and I am sure more will come in the future.

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