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REVIEW

# Dopaminergic neurones: much more than dopamine?

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Midbrain dopaminergic (DA) neurones sustain important physiological functions such as control of motricity, signalling of the error in prediction of rewards and modulation of emotions and cognition. Moreover, their degeneration leads to Parkinson's disease and they may be dysfunctional in other pathological states, such as schizophrenia and drug abuse. A subset of DA neurones has been known for many years to contain releasable peptides such as neurotensin and cholecystokinin. However, recent experimental evidence indicates that the phenotype of DA neurones may be much more diverse, since it is suggested that, under certain conditions, they may also release glutamate, cannabinoids and even serotonin.
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**Abbreviations:** DA, dopaminergic; EPSC, excitatory postsynaptic current; MAOI, monoamine oxidase inhibitor; SSRI, selective serotonin reuptake inhibitor; VGLUT, vesicular glutamate transporter; VMAT, vesicular monoamine transporter

The scientific process is a fascinating mode of operation in which there is an iterative and thorough questioning of seemingly absolute truths. There have been quite a few examples of this lately in neuropharmacology and neuroscience. Does it make sense to label glycine as an inhibitory neurotransmitter whereas it is a coagonist at NMDA receptors? Can GABA be considered as an inhibitory transmitter since it is a major excitatory agent in the perinatal brain? Is the brain really such a 'fixed' structure since it continuously generates new neurones in some areas and this may be part of the therapeutic benefit of antidepressants? Although probably not so radically, the question of the neurotransmitter phenotype of dopaminergic (DA) neurones may have to face quite a significant 'lifting'. Interestingly, such a proposal was already made one decade ago in a very insightful review (Hattori, 1993).

Could Dahlström & Fuxe (1964) have anticipated the fascinating complexity that is gradually emerging from the phenotype of these neurones that they first characterized in the early 1960s with the formaldehyde technique? Major progress in the field of immunology, as well as pharmacological tools and lesion techniques, allowed to extend their findings by showing immunocytochemically that neurones from the A8, A9, A10 groups (among others) indeed are able to synthesize dopamine, but not noradrenaline, and can therefore be labelled as 'dopaminergic' (Bjorklund & Lindvall, 1984).

However, further investigations revealed that the story is much more complex than this.

The 1980s witnessed the emergence of the 'colocalization concept' in these and other neurones. It was demonstrated that a subset of DA neurones also contain cholecystokinin, neurotensin or both, in addition to dopamine, in their presynaptic terminals (Hokfelt *et al*., 1980; Seroogy *et al*., 1988). It was further suggested that the cocktail of neurotransmitters released by DA neurones could vary depending on their firing pattern. Thus, a burst firing pattern appeared to facilitate the release of neurotensin by DA terminals in the rat prefrontal cortex (Bean & Roth, 1991). To many in the field, this was an attractive idea because it could help to explain how these neurones tell their targets that something important is happening. However, knowing the time it should take to replenish neuropeptide vesicles once they have been depleted, it seemed that this could only be a quite rare signal. Moreover, additional studies demonstrated that colocalization of dopamine and cholecystokinin or neurotensin, if any, is quite rare in the human brain (Palacios *et al*., 1989; Savasta *et al*., 1990; Berger *et al*., 1992). Another problem was that all these mediators were found to only activate G-protein coupled receptors, which are known to work relatively slowly, whereas mounting evidence suggested that DA neurones in some instances have to produce rapid signalling. The latter idea was suggested by the influential studies on the physiology of DA neurones in monkeys by Schultz that culminated in his hypothesis that these cells encode errors in the prediction of reward (Schultz & Dickinson, 2000). How could these observations be reconciled?

Recent studies have added layers of complexity to the phenotype of DA neurones and may help envision other modes of communication by these cells.

Work from several laboratories recently revealed that DA neurones may also release glutamate. Progress in this field has been made possible both by a better knowledge of the important proteins involved in glutamate signalling, for example, the large family of membrane transporters and of vesicular transporters (Shigeri *et al*., 2004), and by advances in fluorescent protein technology. Note that there has been very little evidence until recently for a colocalization between a monoamine and an amino acid within CNS neurones

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(Trudeau, 2004) (but see Johnson, 1994). Following early suggestive indications that DA neurones may release glutamate, it was first demonstrated that most DA neurones in sections from rat and monkey brain also immunostain for glutamate and glutaminase (which is involved in the synthesis of vesicular pools of glutamate), but not GABA. Moreover, in culture, DA neurones produced autaptic excitatory postsynaptic currents (EPSCs) that were blocked by CNQX and were inhibited and enhanced, respectively, by quinpirole and sulpiride (Sulzer *et al*., 1998). Similar findings were reported by another group (Bourque & Trudeau, 2000). A real breakthrough occurred with the development of transgenic mice in which DA neurones are fluorescent (by controlling the expression of a yellow fluorescent protein by the promoter of the dopamine transporter). Using brain slices from these animals, Rayport's group was able to convincingly demonstrate that meso-accumbens DA neurones produce a fast CNQX-sensitive EPSC in medium spiny neurones (Chuhma *et al*., 2004). One vesicular glutamate transporter (VGLUT2) has been shown to be expressed by 80% of DA neurones in culture (Dal Bo *et al*., 2004), but there is no clear evidence for its expression by DA neurones in adult animals. Interestingly, there is such evidence for VGLUT3 in serotonergic neurones of the dorsal raphe (Gras *et al*., 2002) and for VGLUT2 in caudal noradrenergic nuclei (but not the locus coeruleus) as well as in adrenergic cell groups (Stornetta *et al*., 2002).

One important question of course is whether glutamate and dopamine release by DA neurones is spatially regulated. This appears to happen in culture. Indeed, it was demonstrated that DA and glutamatergic terminals only partially overlap, with tyrosine hydroxylase-negative and glutamate-positive fibers predominating close to the cell body (Sulzer *et al*., 1998). Moreover, only a subset of tyrosine hydroxylase-positive terminals coexpress VGLUT2 (Dal Bo *et al*., 2004). Congruent with this observation is the fact that in the striatum *in vivo*, about 30% of DA terminals form real synapses, whereas the remaining terminals are devoid of postsynaptic specializations (Descarries *et al*., 1996) and are probably involved in what is known as volume transmission (Agnati & Fuxe, 2000). Future studies will probably advance our understanding of the spatial control of glutamate and dopamine release by DA neurones.

There is now also evidence, both from *in vitro* and *in vivo* experiments, that DA neurones may release cannabinoids that are able to regulate inputs to themselves (Melis *et al*., 2004; Riegel & Lupica, 2004). As is usually the case, cannabinoid release appears to occur from the somato-dendritic compartment. This may turn out to be a characteristic of principal (i.e. output) neurones in many brain areas (Diana & Marty, 2004).

Last, but certainly not least, it was reported this year that, under some circumstances (e.g. treatment with SSRIs or MAOIs), mouse nigro-striatal DA neurones can release serotonin from their terminals (Zhou *et al*., 2005). Indeed, serotonin can be taken up at DA terminals by the dopamine transporter (identified pharmacologically by its sensitivity to GBR12909). Quite fascinatingly, serotonin may somehow compete with dopamine for the uptake by the vesicular monoamine transporter that is expressed by these neurones (VMAT2) into synaptic vesicles, thereby proportionally reducing the amount of dopamine which is released. Although it remains to be seen whether this can also be found in the clinical setting, this observation is very interesting. It should be pointed out that interactions between aminergic neurones have already been described at many levels (besides the heteroreceptor level), including transporters (Moron *et al*., 2002) and receptors (Malenka & Nicoll, 1986; Cornil *et al*., 2002). However, such an 'intimate' interaction as the one described by Zhou *et al*. had not been directly demonstrated before, despite some preliminary suggestive evidence (Stamford *et al*., 1990; Suarez-Roca & Cubeddu, 2002).

Now the question arises: is it still appropriate to simply label these neurones as 'dopaminergic' (although for didactic purposes, it is probably still a good idea!)? Can we perhaps view these neurones as having a more complex role in the brain than 'simply' releasing dopamine (even if it is clear from the treatment of Parkinson's disease that the latter is a very important one)?

I believe that this question is now relevant, both from the neurobiological standpoint, but also in the context of the pharmacological manipulation of the DA system. Of course, important questions remain to be addressed: how plastic is the neurotransmitter phenotype of these neurones, both in physiological and pathophysiological conditions? Is the cocktail of neurotransmitter released different in the various subsets of midbrain DA neurones?

Taken together, these observations are in favour of a hypothesis in which these neurones are indeed able to work on different time scales, as suggested by Schultz in his plenary lecture at last year's Society for Neuroscience meeting (also see Lavin *et al*., 2005).

What are the potential consequences of these recent developments in the field of Pharmacology? If it turns out that the neurotransmitter phenotype of DA neurones varies in pathological conditions, say levodopa-induced dyskinesias, new therapeutic possibilities for such conditions may emerge. More broadly, the kind of intimate interaction between neurotransmitter systems described by Zhou *et al*. may help explain how antidepressants with different mechanisms of action can have therapeutic effects that are clinically very similar. It remains to be seen whether uptake and release of serotonin by DA neurones is a prerequisite to the therapeutic effect of antidepressants. This question might be answered by brain imaging.

Finally, there is also the suggestion (Zhou *et al*., 2005) that the release of serotonin by DA neurones may somehow contribute to the so-called serotonin syndrome, a potentially life-threatening condition which is due to an excess of serotonin in some regions of the brain, usually during a combined treatment with an SSRI and another drug which increases the brain serotonin concentration (Boyer & Shannon, 2005).

Whatever the future experiments will tell us, this area of research is currently a very exciting one.

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