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The discovery of dopamine deficiency in the parkinsonian brain

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Summary. This article gives a short historical account of the events and circumstances that led to the discovery of the occurrence of dopamine (DA) in the brain and its deficiency in Parkinson's disease (PD). Some important consequences, for both the basic science and the patient, of the work on DA in the PD brain are also highlighted.

Early opportunities

In 1951, Wilhelm Raab found a catecholamine (CA)-like substance in animal and human brain (Raab and Gigee, 1951). He knew that this CA was neither noradrenaline (NA) nor adrenaline; today, we know that it was, at least in part, dopamine (DA). Raab examined its regional distribution in the brain of humans, monkeys and some "larger animals", and found highest levels in the caudate nucleus. He found no changes of this CA in the caudate in 11 "psychotic" patients. He did not try to look for this compound in the caudate nucleus of patients with Parkinson's disease (PD).

In 1952, G. Weber analyzed brains of patients with PD, obtained postmortem, for cholinesterase activity (Weber, 1952). He found a reduction of the enzyme activity in the putamen, and hypothesized about the significance for PD. Had Weber known of Raab's study published the year before, he might have measured Raab's CA-like compound in his PD postmortem material. In his report, Weber does not refer to Raab's study.

In 1952–1954, Marthe Vogt performed her landmark study of the regional distribution of NA and adrenaline in the brain of the dog (Vogt, 1952, 1954). She isolated the amines from brain tissue extracts by paper chromatography and eluted the corresponding "spots" for (biological) assays. Marthe Vogt was well aware of Raab's work. However, for practical reasons, she did not stain the CA (with ferricyanide) on the chromatograms of regions that contained little NA, such as the caudate; thus she let pass the opportunity of detecting DA's presence in the brain and its striatal localisation.

Setting the stage for the DA/PD studies

In August 1957, Kathleen Montagu reported on the presence of DA, identified by paper chromatography, in the brain of several species, including a whole human brain (Montagu, 1957). In November 1957, Hans Weil-Malherbe confirmed this discovery and examined DA's intracellular distribution in the rabbit brain stem (Weil-Malherbe and Bone, 1957). Neither he, nor Montagu, offered any speculations on the physiological role of brain DA. At the same time as Weil-Malherbe, in November 1957, Arvid Carlsson observed that in naïve and reserpine treated animals "*3,4-dihydroxyphenylalanine caused*

central stimulation which was . . . markedly potentiated by iproniazid" (Carlsson et al., 1957). He concluded that the study "*supports the assumption that the effect of 3,4-dihydroxyphenylalanine was due to an amine formed from it"* – leaving the question of whether this amine was NA or DA, unconsidered. In the Fall of 1957, a few weeks before Carlsson's report, Peter Holtz published observations on, *inter alia*, L-dopa's central stimulant and "awakening" (from hexobarbital anesthesia) effects, and clearly suggested, apparently for the first time, that this could be due to the accumulation of "*the dopamine formed in the brain from L-dopa"* (Holtz et al., 1957). (Raab, in 1951, was the first to observe increased brain levels of his CA-like substance after i.p. L-dopa; but he does not mention any behavioral L-dopa effects [Raab and Gigge, 1951].)

Holtz's conclusion was soon confirmed in two biochemical studies. In February 1958, Carlsson reported that reserpine depleted, in addition to NA and serotonin, brain DA, and L-dopa replenished it while causing central excitation (Carlsson et al., 1958). In May 1958, Weil-Malherbe obtained, independently, the same biochemical results in a well documented study (Weil-Malherbe and Bone, 1958). Neither Carlsson nor Weil-Malherbe ventured any explicit statements about brain DA's possible physiological role or its involvement in the reserpine syndrome.

More than a year before these first brain DA studies, in the Fall of 1956, Blaschko had already proposed that DA – until then seen as being merely an intermediate in the biosynthesis of CA – had "*some regulating functions of its own which are not yet known"* (Blaschko, 1957). In early 1957, Hornykiewicz, in Blaschko's Oxford laboratory, tested this idea experimentally. He analyzed DA's vasodepressor action (in the guinea pig) and proved that DA had actions distinct from NA and adrenaline and thus qualified as a biologically active substance in its own right; L-dopa behaved exactly like DA (Hornykiewicz, 1958). In 1958, Hornykiewicz (now back in Vienna) examined (in the rat) the central actions of several substances, including the parkinsonism-inducing chlorpromazine and bulbocapnine, as well as cocaine and MAO inhibitors, and showed that only the latter affected (increased) the levels of brain DA (Holzer and Hornykiewicz, 1959).

Marthe Vogt, in her 1954 NA study in the dog brain, inferred NA's possible role in brain function from the amine's specific distribution pattern. In January 1959, Åke Bertler and Evald Rosengren, patterning themselves on Marthe Vogt's NA study, published a study, also in the dog, on the regional distribution of brain DA (Bertler and Rosengren, 1959a); a few weeks later, Isamu Sano reported on DA's regional distribution in the human brain (Sano et al., 1959) (followed by Bertler and Rosengren, 1959b). Both research groups found that DA was mostly concentrated in the nuclei of the basal ganglia, especially caudate and putamen. Bertler and Rosengren (1959a) concluded that their "*results favour*[ed] *the assumption that dopamine is connected with the function of the corpus striatum and thus with the control of movement"*; and Sano "*considered DA to function in the extrapyramidal system which regulates the central motoric function"* (Sano et al., 1959). Although Bertler and Rosengren pointed out DA's possible involvement in reserpine parkinsonism, neither they nor Sano suggested the possibility of striatal DA being directly involved in diseases of the basal ganglia.

DA is severely reduced in PD striatum

Several eyewitness accounts have recently been written about the historical events and consequences of the discovery of the DA deficiency in PD (Sourkes, 2000; Hornykiewicz, 2001a, b, 2002a, b).

Early in 1959, Hornykiewicz, aware of DA's localisation in the basal ganglia, started

a study on DA in postmortem brain of patients with PD and other basal ganglia disorders. He and his collaborator Herbert Ehringer analyzed the brains of 17 adult non-neurological controls, 6 brains of patients with basal ganglia disease of unknown etiology, 2 brains of Huntington's disease, and 6 Parkinson brains. Of the 14 cases with basal ganglia disease, only the 6 PD cases had a severe loss of DA in the caudate and putamen (Ehringer and Hornykiewicz, 1960). Ehringer and Hornykiewicz concluded that their observations "*could be regarded as comparable in significance* [for PD] *to the histological changes in substantia nigra"* . . . so that "*a particularly great importance would have to be attributed to dopamine's role in the pathophysiology and symptomatology of idiopathic Parkinson's disease"*. This discovery was published in December 1960. Ever since, it has provided a solid, rational basis for all the following research into the mechanisms, the causes, and new treatments of PD.

It is interesting to note that in none of the brain DA and/or L-dopa studies preceding the Ehringer and Hornykiewicz 1960 paper, is there any hint to be found that such a study should be done. The first such suggestion was made in an article from Montreal, submitted for publication end of November 1960, reporting on reduced urinary DA in PD patients. The authors concluded that future investigations should "*include analysis of the catecholamine content in the brains of patients who have died with basal ganglia disorders"*, so as to "*help determine whether the concentration of cerebral dopamine itself undergoes major changes"*. The article was published in May 1961; a "note added in proof" informed the readers that the suggested study has, in the meantime, been done (Barbeau et al., 1961).

The fact that the Montreal group quoted the paper from Vienna so soon after it was published on December 15, 1960, deserves a comment. This article was written in German and published in a German language journal. Theodore Sourkes, the leading biochemist of the Montreal group, must have read it almost immediately after it came out. He contacted Hornykiewicz about this article by letter dated February 10, 1961. For the Vienna discovery, there were, obviously, neither language nor information transfer barriers. This was opposite to what happened to a (lecture) overview article of Sano, published in Japanese in 1960. Independently from Hornykiewicz, Sano had analyzed the brain of a single PD patient, but was "*reluctant to speculate, from that single experience* [low putamen DA] *about the pathogenesis of Parkinson's disease"* (Sano, 1962). The publication remained unnoticed until it was recently reprinted in English translation (Sano, 2000).

The question arises: Why did none of the pioneers of the early brain DA research think of studying the PD brain? It appears that the main reason was their too exclusive preoccupation with the central effects of reserpine. This is surprizing because even then it was obvious that reserpine, like most pharmacological animal models, was not a perfect centrally acting drug; it depleted, to the same degree as DA, also the brain NA and serotonin, making a clear decision about the relative importance of these changes impossible. The exclusive "fixation" on reserpine made leading monoamine researchers of that period overlook the most obvious, that is, PD as the ultimate "brain DA experiment of Nature".

Two practical consequences

Inaugurating the nigrostriatal DA pathway

When the DA deficiency in PD was discovered, nothing was known about DA's cellular localisation in the brain. In Huntington's disease, Ehringer and Hornykiewicz (1960) had found normal striatal DA. Since in Huntington's disease there is a severe loss of striatal neurons accompanied by marked gliosis, the normal striatal DA suggested that the amine was probably contained in terminals of fibre tracts originating outside the striatum. Rolf Hassler

had proved, back in 1938, that in PD, loss of the substantia nigra compacta neurons was the most consistent pathological change (Hassler, 1938). Thus, in 1962, Hornykiewicz started a study of the substantia nigra in 10 PD brains. The outcome of such a study was by no means certain. Hassler himself rejected the possibility of a nigro-striatal connection (see page 869 in: Jung and Hassler, 1960); and Derek Denny-Brown declared, in 1962, that "*we have presented reasons against the common assumption that lesions of the substantia nigra are responsible fo parkinsonism"* (Denny-Brown, 1962). In his study, Hornykiewicz found markedly reduced nigral DA, similar to the DA loss in the striatum. In the report published in 1963, Hornykiewicz concluded from his observation that "*on the other hand, cell loss in the* [PD] *substantia nigra could well be the cause of the dopamine deficit in the striatum"* (Hornykiewicz, 1963).

At the time of Hornykiewicz's DA/substantia nigra study, two research groups were already trying to tackle the question of brain DA's cellular localization. In Montreal, Poirier and Sourkes were using electrolytic brain lesions, in the primate; in Sweden, Fuxe, Dahlström (and others) were applying, in the rat, the just developed CA histofluorescence method. A year after Hornykiewicz published his study, each of the two research groups was able to report on the existence of a DA-containing nigrostriatal connection. Both groups referred, in their first publications, to Hornykiewicz's 1963 nigral DA study (Andén et al., 1964; Dahlström and Fuxe, 1964; Poirier and Sourkes, 1965). This contribution to the discovery of the nigrostriatal DA pathway had for Hornykiewicz yet another consequence. Several years later, Hassler wrote him a letter in which he expressed his candid opinion on the nigrostriatal DA pathway. He wrote: "*I believe that your interpretation of your observations does not agree with many known facts, this being so because you accept the American* [?!] *opinion about the direction of the nigrostriatal connections. I believe that all your observations can be equally well, or even better, explained by the striatonigral direction* [of that pathway]*"* (Hassler, 1967).

L-dopa for the PD patient

The discovery of the severe striatal DA deficiency in PD had also a far-reaching clinical consequence. Hornykiewicz immediately took the step "from brain homogenate to treatment" and asked the neurologist Walther Birkmayer to do clinical trials with i.v. L-dopa. After a delay of eight months, in July 1961, Birkmayer injected 50–150 mg L-dopa i.v. in 20 PD patients, most of them pretreated with an MAO inhibitor. The first report, published in November 1961, conveys, even today, the excitement about what since has been called "the dopamine miracle"; it reads as follows:

The effect of a single i.v. administration of L-dopa was, in short, a complete abolition or substantial relief of akinesia. Bed-ridden patients who were unable to sit up; patients who could not stand up when seated; and patients who when standing could not start walking, performed after L-dopa all these activities with ease. They walked around with normal associated movements and they even could run and jump. The voiceless, aphonic speech, blurred by pallilalia and unclear articulation, became forceful and clear as in a normal person. For short periods of time the patients were able to perform motor activities which could not be prompted to any comparable degree by any other known drug. (Birkmayer and Hornykiewicz, 1961).

Simultaneously with, and independently from, the trials in Vienna, Sourkes and Murphy, in Montreal, proposed to Barbeau a trial of oral L-dopa. They observed, with 200 mg L-dopa, an amelioration of rigidity that "*was of the order of 50 percent"* (Barbeau et al., 1962). Interestingly, Sano in his overview in 1960 also mentioned that he

had injected 200 mg L-dopa i.v. in two patients; however, he did not evaluate the effect clinically, being "*more interested in subjective complaints"* (Sano, 1962). Sano concluded that "*treatment with dopa has no practical value"* (Sano, 2000).

Today, especially thanks to Cotzias's introduction of the high dose oral treatment regimen (Cotzias et al., 1967), L-dopa is recognized as the most powerful drug available for PD. As Sourkes very aptly expressed it, the discovery of L-dopa "*proved to be the culmination of a century-and-a-half search for a treatment of Parkinson's disease"* (Sourkes, 2000).

Despite the unprecedented success, doubts were expressed about L-dopa's "miraculous" antiparkinson effect. Many neurologists suspected a placebo effect of the i.v. injected L-dopa, ignoring the fact that Birkmayer and Hornykiewicz (1962) had described, already in 1962, the ineffectiveness of i.v. injected compounds related to L-dopa, such as: D-dopa, 3-O-methyldopa, DA, D, L-dops, and also 5-HTP. This should have convinced the doubters that the L-dopa effect could not have been a placebo effect.

Especially counterproductive were various statements by some rather prominent brain scientists. Thus, some claimed that "*the actions of DOPA and DOPS* [the direct precursor of NA] *were similar"*, cautioning that "*dopamine can activate not only its own receptors* [in the brain], *but also those of noradrenaline, and vice versa"* (Carlsson, 1964, 1965); others felt that "*the effect of L-dopa was too complex to permit a conclusion about disturbances of the dopamine system in Parkinson's disease"* (Bertler and Rosengren, 1966), still others compressed all their doubts in the terse phrase that L-dopa "*was the right therapy for the wrong reason"* (Ward, 1970; Jasper, 1970); and, finally, there was the statement that "*since L-dopa floods the brain with dopamine, to relate its* [antiparkinson] *effects to the natural function of dopamine neurons may be erroneous"* (Vogt, 1973). These and similar critical statements diminished the status of L-dopa as a specific DA replacing agent and put in doubt the very concept of DA replacement in PD.

Viewed against the background of the initial skepticism, today's opinion has substantially changed, as reflected, for instance, in a recent "Editorial":

The identification of the dopaminergic deficit in Parkinson's disease and the development of dopamine replacement therapy by Hornykiewicz and his contemporaries profoundly influenced research into Parkinson's disease, and perhaps even all neurological disorders. This is especially true for Alzheimer's disease, in which current cholinergic therapy is the intellectual heir of dopamine replacement therapy for Parkinson's disease. (Hardy and Langston, 2004).

Thus has theoretically based research led, in an amazingly straight line, to very practical results. As Immanuel Kant, that eminent philosopher of the Age of Enlightenment, put it some 200 years ago: "There is nothing more practical than a sound theory".

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