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Articoli/*Articles*

DANIEL BOVET AND HIS ROLE IN THE DEVELOPMENT OF PSYCHOBIOLOGY

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SUMMARY

One hundred years since his birth, fifty years after his Nobel achievement, Daniel Bovet still emerges as one of the key figures of both pharmacology and psychobiology, the biological and evolutionary roots of behaviour. The life and scientific activities of Daniel Bovet (1907-1992) are closely linked to the 'golden years' of pharmacology, the exceptional development of this science from the end of the 1930s to the 1960s. Later on, from the 1960s to the end of his scientific career, Bovet entered a new field, psychobiology, through the study of the effects of drugs active on the nervous system and their effects on behaviour. This approach led him to explore different aspects of the biology of behaviour, namely the role of individual differences, the genetic determinants of behaviour and their implications on learning and memory. It is therefore evident that the range of his scientific activity has been very broad, a fact difficultly conceivable in years of extreme specialization.

Bovet won the 1957 Nobel Prize in Physiology and Medicine for his discovery of drugs that block the actions of specific neurotransmitters. He is best known for his discovery in 1937 of antihistamines, used in allergy medication: however, his contribution is very broad and ranges from chemotherapy to the sulphonamide drugs, the pharmacology of the sympathetic nervous system, the therapy of allergic conditions, the synthesis of antihistamines, curare and curare-like

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drugs and the use of curare as an adjuvant to anaesthesia, different aspects of the pharmacology of the central nervous system and in the last years of his career behaviour genetics and the effects of drugs on learning and memory.

There are three main periods in the career of this scientist: 1. from 1929 until 1947 he worked at the Pasteur Institute in Paris, then under the direction of Professor E. Roux, where he became Chief of the Laboratory of Therapeutic Chemistry where he was a collaborator and later a successor of Ernest Fourneau. 2. From 1947 to 1963 he worked in Rome at the Istituto Superiore di Sanità, then directed by Domenico Marotta. 3. From 1963 to 1982 after leaving the Istituto he moved to the University of Sassari, an subsequently to Rome and the Laboratory of Psychobiology and Psychopharmacology of CNR, the Italian National Research Council: it is mostly during this period that he privileged the study of psychobiology, a field initiated during the last years at the Istituto Superiore di Sanità.

1. *From the Institut Pasteur to Rome*

The backbone of Bovet's research rests on his work at the Pasteur Institute where hi and his co-workers, (among them his wife Filomena Bovet Nitti, and her brother, the bacteriologist F. Nitti) succeeded in discerning the links between the most disparate natural or synthesized molecules, in fathoming their physiological and pharmacological mechanisms. His work on the relationships existing between the structure of a molecule and its function was something like the creation of a great fresco encompassing adrenaline and other sympathomimetic agents, the derivatives of dioxane, the amphetamines, the derivatives of lysergic acid, and so on. This enormous labour of research and classification made it possible to 'follow the tracks' of molecules resembling each other but, at times, producing opposite effects at the level of the nerve receptors, which were still unknown at the time. The task was described by Bovet and Bovet Nitti in

Structure et activité pharmacodynamique des médicaments du système nerveux végétatif (Karger, Basel, 1948), referred to jokingly by their co-workers as the Bible. Years after its publication, this Bible was still being used to track down molecular affinities and antagonisms of such complexity that the author of the monumental treatise was himself often obliged to consult it in order to pinpoint this or that minor molecule among the thousands and thousands studied by the group created by Ernest Fourneau at the Pasteur Institute.

As anticipated, in 1947, Bovet accepted an invitation made by Domenico Marotta, director of the Istituto Superiore di Sanità, and moved to Rome with his wife Filomena to take charge of a large pharmacological laboratory which, like that at the Pasteur Institute, had been given the name of 'Therapeutic Chemistry'. The work of his new research team focused primarily on curare and on a vast range of substances acting on the central nervous system and on cerebral circulation: these steps marked a transitional stage on the path towards psychopharmacology and the biology of behaviour. When I joined Bovet's team in the early 1960s, he had been in charge for over a decade of the great laboratory at the Istituto di Sanità, its various sections including chemistry, physiology, the electrical activity of the brain, cerebral circulation and behaviour. Having been awarded the Nobel Prize for medicine a few years earlier (1957) 'for discoveries regarding synthesized products that block the effects of a number of substances which form within and act upon the organism, in particular on the blood vessels and the musculature of the skeleton', Bovet was still at the peak of his scientific creativity, working in a laboratory crammed with foreign guests where it was possible to collaborate with other interesting research groups. Sir Ernst Boris Chain, another Nobel Prize winner, was also at the Istituto di Sanità as head of a team working on antibiotics and protein synthesis. Rita Levi Montalcini, later to win the Nobel Prize for her work on neurobiology, was a recent arrival. Numerous other scientists of real merit,

from physicists to virologists, had also been drawn to the Institute by a climate of collaboration and a concentration of economic and scientific resources seldom found in Italy.
I met Bovet in his office, from which one glimpsed the laboratory and the shining glass manometers of the Warburg apparatus then used by his wife to gauge interactions between cholinergic enzymes, drugs and nervous substrata through microvolumetric measurement of the gases. Though I was young and had only just graduated, Bovet spoke to me at length as though we were of equal scientific standing. His courtesy was somewhat disconcerting for anyone accustomed to the strongly hierarchical environment of Italian medicine at the time, where professors seldom had any contacts with their younger students or filtered them carefully through a complex pyramid of collaborators. Bovet's courtesy and openness were part of his charm. The absence in the laboratory of scientific hierarchies connected with position or age made his scientific activities similar in some respects to those of the English-speaking world. Bovet's group was rather large and ranged from an electrophysiological approach (Vincenzo Longo) to the study of cerebral circulation (Amilcare Carpi), synthesis of biological molecules and curare (Vittorio Rosnati e Giovan Battista Marini Bettòlo), screening of drugs (Bruno Silvestrini) and the artificial selection of two lines of rats (the Roman high and Roman low) characterized by opposite performances in an automated avoidance task (Giorgio Bignami). In addition to these groups, a number of foreign guests worked in the laboratory: many of them from South America (due to the good relationships between Bovet and Carlos Chagas and their shared interest in curari) but also from France, the U.K. such as the very young Stephen P.R. Rose or the U.S.A, such as James L. McGaugh, then a postgraduate student already interested in the relationships between brain and behaviour.

2. *Measuring behaviour: the role of instrumentation*

A common problem for many physiological and pharmacological laboratories at the beginning of the 1960s was the modernization of various classical techniques and methods, some of them many decades old: for example, tracking the contraction of an isolated intestinal strip or the activity of an isolated heart required the activation of a system of levers and the recording on a the smoked paper of a revolving drum where the last lever, the "pen", left a white trace on lampblack: the process of "smoking the drums" required the ability of a skilled technician and I remember a room where dozens of black drums waited to be brought to the laboratory. Bovet was aware that some of these techniques would become obsolete and that a discontinuity with the tradition was necessary. An important update was the purchase of different Grass polygraphs, where it was possible to amplify and simultaneously record many physiological parameters: these machines were considered so important that in the cold-war years there was a restriction to sell them to eastern countries. However, the biological bases of behaviour were becoming Bovet's main interest: he considered behaviour as a phenotype like others, at least in terms of its recording and quantification. Thus, he felt that researchers should not rely on mere observational measures, such as scoring the movements of an animal in an open field or the path covered to attain the goal-box of a maze. He decided that in order to study learning and memory in animals it was necessary to depend on a constant motivation and on clear-cut scores: this brought him to chose the shuttle-box or "Warner cage" as a the choice task and to develop automated programs and records. He adopted a Crouzet cam programmer were it was possible to set the lengths of the conditioned stimuli (light or sound), the length of the unconditioned stimulus (generally a light electric shock delivered through the metal bars of the floor of box the where the animal was tested), the interval between trials and the length of the conditioned avoidance session. The apparatus to test the behaviour of mice and rats, the shuttle-boxes, was built by a small Italian company (Ugo Basile today one of the world leading manufacturers of instruments for physiological and pharmacological research): after the production of the first prototype different batteries, each of eight boxes, were manufactured. The same equipment was later used in the University of California in Los Angeles, when Bovet and myself moved in 1965 to work at the Brain Research Institute, and later on to the University of Sassari and finally to Rome. For many years these automated behavioural apparatuses have been a unique opportunity to study the behaviour of large groups of animals in an objective way: through their use it has been possible to trace learning curves, to assess the effects of drugs on behaviour and to show that learning and memory could be quantified like any other phenotype. In addition to these automated shuttle-boxes, other equipments were ideated or improved such as activity wheel to measure circadian rhythms, automated water mazes, visual discrimination apparatuses and so on: though this list of machinery may sound trivial, it was thanks to these technical improvements that it has been possible to make subtle and reliable behavioural analyses.

3. *The decline of behaviourism and the rise of neurosciences and psychobiology*

Paradoxically, I believe that the political crisis that affected two key scientific institutions in the middle of the 1960s contributed to orient Bovet's interests in the direction of behaviour. In fact, since he was forced to resize his laboratory into a smaller scale, he gave up other research areas such as electrophysiology and the facilities to synthesize new drugs and neurochemicals which required a very extensive research group: after the deep crisis that affected the Institute it would have been very difficult to rebuild a large laboratory and to recreate a versatile research team. In 1964 a real earthquake affected many scientific institutions: first, Felice Ippolito, secretary of CNEN (Comitato Nazionale Energia Nucleare) was arrested and the Italian nuclear programme brought to a halt; in the same year Domenico Marotta, director of the Istituto di Sanità, was also arrested in the frame of an obscure business of political rivalry and career-hunting that led to traumatic intervention by the magistrates and brought research activities to a virtual standstill for years. When the scandal broke and brought down the Institute's director, Bovet and others stood up for Marotta. He could scarcely fathom the political and bureaucratic coils in which the Institute was being enmeshed and was deeply upset by the irrational nature of the situation, unlike the sanguine Chain, whose vehemently outspoken diatribes echoed through the corridors). Sir Ernst Chain left Italy for the Imperial College in London. Levi-Montalcini increased her working periods in St. Louis an later founded a laboratory in Rome financed by the National Research council. Bovet, like others, guessed that life would be hard for the Institute, that the scandal would drag on for years and that life there would long be dominated by chronic rivalry and in-fighting. He thus decided to pack his bags and seek a new position, but quite underestimated the resistance his candidature would provoke in Italy's academic corporation. The author of hundreds of scientific publications and winner of the Nobel Prize with degrees honoris causa from numerous universities had a hard time trying to win a chair in pharmacology because of his 'lack of teaching experience'. However, in 1964 he was appointed professor of pharmacology at the University of Sassari in Sardinia, where he remained until the end of the 1960s, alternating his research activities between Sassari and the Brain Research Institute of the University of California in Los Angeles (UCLA).

Undoubtedly, apart from being a Nobel prize winner, a factor that contributed to Bovet's new position at UCLA, was his search for automated, reliable behavioural measures. UCLA, with its Brain Research Institute, founded in 1959 by Dr. Horace W. Magoun and Dr. Donald B. Lindsley, where it was also located the Space Biology Laboratory directed by W. Ross Adey, extremely advanced in terms of automation and computer facilities, was a very interesting site to start a new scientific adventure. The person who gave a hand to Bovet and contributed to setting up a behavioural laboratory at UCLA was Alexander Kolin, a biophysicist expert in electromagnetic flow measures interested in Bovet's studies on cerebral circulation and, more generally, in his automated apparatuses. Kolin, who was also very gifted in making up new gadgets and machineries and admired the behavioural equipment developed at the Istituto Superiore di Sanità, introduced Bovet to Sherman Mellinkoff, dean of UCLA School of Medicine, and in a matter of a few weeks a laboratory was ready at the Brain Research Institute, together with substantial financial support: this seemed to us a miracle in times in which the Italian science was facing a crisis and entering a grey period. Bovet accepted Mellinkoff's invitation with great enthusiasm, above all as he had for some time been engaged on research into behavioural genetics, using inbred strains of mice. These were then quite rare in Europe but readily available in the USA, where they were used in radiobiological research at Oak Ridge and immunogenetic studies at the Jackson Laboratory in Bar Harbour. He thus left for Los Angeles and worked for some years on the effects of cholinergic and adrenergic drugs on behaviour. Also involved was James L. McGaugh of the University of California at Irvine, who had worked with Bovet at the Institute in Rome.

In the middle of the 1960s, the behavioural sciences in the USA were just emerging from a strict behaviourist approach, influenced by the charismatic figure of Burrhus F. Skinner (1904-1990) and by his school. Behaviourism was mostly environmentally and functionally oriented, thus denying the importance of any research on the structure and function of the brain (therefore it was of no use opening the "black box" –the brain- to understand behaviour) and the role of biology, genetics, and individual differences. To behaviourists, still exerting in the 1960s a strong ideological and academic hold in the USA, it was basically similar to study the behaviour of a pigeon, a rat or a human being, since they considered that the laws of behaviour were substantially identical for all animals. Thus, behaviourists were not interested in individual differences, comparative analyses, the evolutionary roots of behaviour nor, of course, in penetrating neural circuits and in tracing brain-behaviour correlations. This position was attacked by the well known psychologist Frank Beach (1911-1988), author of a seminal article entitled "The snark was a boojum" in favour of an integration of ethology and comparative psychology. At UCLA and the BRI a number of "neuroscientists" (a term just created by Francis O. Schmitt), such as Horace Winchell Magoun (1907-1991, who discovered the reticular formation together with Giuseppe Moruzzi), John Douglas French (1911-1989) a distinguished neurosurgeon and investigator, Samuel Eiduson (1918-2007) a key figure in the study of the role of serotonin in the brain, and many others favoured a strong psychobiological evolutionary approach and contributed, together with Bovet, to a real change of direction in respect to the usual behaviourist approach.

4. *Assessing behavioural individuality*

The most significant approach in this direction came from Bovet's studies on the effects of nicotine on behaviour, a research in line with his classical work on cholinergic and anticholinergic agents. The first results on the action of nicotine on behaviour indicated that the effects exerted by nicotine were stimulating or depressant depending on the genetic background of the animals, a fact that showed how important individual differences are in terms of drug reactivity. Ever since that time Bovet's (and my) interest in psychopharmacology has been filtered through a genetic approach. The term "psychopharmacogenetics" had still to be invented, although several different research groups were already interested in the problem of individual reactivity to drugs from both a quantitative and a qualitative point of view. A first screening of the behavioural effects of nicotine and nicotinic agents revealed clear-cut differences when different mouse strains were considered: contrasting reactions characterized various inbred strains that very soon appeared to be an interesting model for understanding the neurochemical and neurophysiological correlates of the observed divergent effects. Thus, Bovet used neuroactive drugs as probes to explore the nervous system and to gauge the variability of nerve structures and behaviour. These studies in psychopharmacology and psychopharmacogenetics were soon to be flanked by studies into behavioural genetics based on the use of selected strains of rodent and inbred strains of mice. One of the most interesting results of these studies involves the demonstration of the role played by genetic factors in conditioning certain aspects of learning and memory:

The findings reported here suggest that there are at least two reasons for using inbred strains in psychobiology: (i) the extreme behavioral homogeneity of the individuals belonging to the same strain, and (ii) the characteristic differences in behavioral traits of each strain. These inbred strains provide the psychobiologist with unlimited groups of individuals presenting a homogeneous adaptive behaviour. This availability is particularly important in view of previous difficulties and problems surrounding the establishment of learning and retention curves in laboratory animals[1].

Since 1965 the approach of Bovet's group became genetically-oriented in order to assess the effects of a number of cholinergic, noradrenergic and later dopaminergic agents in terms of their action on different genetic backgrounds, that is to say, in terms of the qualitative and quantitative receptor patterns and the neuronal systems involved. As already noted, drugs were interesting mainly as tools for scanning the brain and behaviour rather than because of or in addition to their possible therapeutic effects. Year after year, experiment after experiment, it was evident that in contrast to behaviouristic tenets, the brains of different species, strains or individuals were characterized by a huge diversification of expression of both nervous structures and behaviours. For example, a large variability in the levels, turnover and cerebral distribution of neurotransmitters responsible for different patterns of arousal and energy-deployment mechanisms was described. Intraspecific variations in brain chemistry and neurotransmitter turnover, in addition to a number of morphological measures involving brain size, hippocampal volume, hypothalamic or limbic organization, were evident in mice during both development and adult age. When considered in terms of learning and memory, these neurochemical, neurophysiological and neuroanatomical variations result in both clear-cut memory differences, a fact that does not necessarily imply that "basic" memory mechanisms must be different. Many findings indicate that the neurobiological mechanisms involved in memory consolidation are essentially similar within and across species since they are based on almost identical functional or structural changes at the neuronal level. However, a number of sensory, arousal, attention, or emotional processes may impair or improve the different "basic" mechanisms of memory, that is to say, they exert a modulation on memory processes. It is this modulation that is affected by different brain chemicals and by a number of drugs that can exert profound effects.

5. *Genetics and behaviour*

These studies, initiated at UCLA were in fact to continue in Rome, where Bovet was invited in 1969 by the Faculty of Natural Sciences as Professor of Psychobiology, a new discipline for Italy, where idealistic schools and attitudes still predominated in psychology.

In Rome, Bovet created the Laboratory of Psychobiology and Psychopharmacology of the Italian National Research Council, which he directed for ten years, still continuing his activities in the field of behaviour genetics until he retired in 1982. By following a classic Mendelian genetic approach, Bovet demonstrated that some behaviour patterns possess important biological components in that they depend on specific differences in the nervous system at the level of neurobiological structures, from nerve receptors to the extension of some cerebral nerve nuclei. He proposed that research in the neurosciences and psychobiology should be based on certain particular strains characterized by opposite phenotypes:
These three inbred strains of mice and their F1 hybrids seem to be a very useful model for a genetic approach to the biological aspects of learning. The results of different biochemical estimates suggest in part that these lines and their crosses not only differ in behaviour but also in some critical brain chemicals. Mandel and his group found large regional differences between these strains when their cholinergic and adrenergic levels and turnover were measured. Also the brain level of dopamine and cyclic AMP were found to be different in these strains[2].
This highly innovative genetic approach implied that different behavioural aspects were regarded as phenotypes, thus shedding light inside the famous 'black box'. The approach was also connected with the important topic of the variability of neurobiological structures due to both genetic and epigenetic factors, since taken up by Gerald Edelman in his theory of 'neuronal Darwinism'. Later on, with the development of molecular biology, behaviour genetics found new horizons through the use of transgenic and knockout animals (mostly mice). The Mendelian approach, based on crosses of inbred strains, is today outshined by more selective and powerful tools but these rest on those studies demonstrating that many behav-

ioural patters are characterized by neurobiological roots that it is possible to identify and, in some instances, manipulate.

There is a final aspect of the rise of psychobiology that should be considered: not only at its beginnings the biological approach had its fierce opponents in the scholars of behaviourism but it also encountered a strong ideological hostility from the more radical groups at the beginning of the 1970's. It was feared that it would legitimate social or class differences by implying that "all was in the genes" rather than in the culture. In Italy, where political life was somewhat radicalized, many psychiatrists, psychologists and even neuropharmacologists tagged the genetic approach as being conservative and likened it to a kind of rough reductionism. A similar position was held on a different ground by a number of philosophers and sharp dualists in favour of a completely immaterial mind: I still remember a meeting in Luzern, Switzerland, in which John Eccles manifested his harsh opposition to the idea that also individual neural differences might result in behavioural differences, though the subject of my talk was restricted to the behaviour of mice. Despite these radical positions, the use of drugs as tools for scanning brain functions and a genetic dissection of behaviour has proved one of the most important approaches to modern neuroscience. If today's theories of mind reflect empirical evidence rather than abstract positions, it is also due to the role of psychopharmacology and behaviour genetics. Today's students do not fully appreciate that, less than four decades ago, our knowledge of the brain was limited to a small number of neurochemical, neurophysiological, pharmacological and clinical data. Thanks to several approaches based on healthy reductionism, such as those of the physiologists who considered that what was known at the peripheral (nervous) level would prove useful at the central level, we know much more about cognition, emotion and about the drugs that affect these processes.

Bovet was a realist aware both of what could be done in a given situation and of what would instead cause too many problems. His scientific choices also reflect this aspect, i.e. his ability to select areas of research where it was possible to compete with groups far better endowed in terms of funds, organization and human resources not head-on but through innovation, scientific bricolage and creativity. In any case, it was essential for Bovet to press forward and overcome all possible obstacles in that science was central to his system of values. His autobiographical book[3] ends with the following words:
My generation unhesitatingly followed the enlightened conception of science as a source of progress, as being good by definition, and my opinion remains unchanged despite the terrible applications that have marked our century in the field of physics. While it does not appear possible as yet to conceive of a consistent and flawless system of scientific thought at the levels of logic and ethics, it would in any case make no sense to believe that wisdom lies in a deliberate return towards the irrational. Though our knowledge may be fragmentary, though research may at times have been a source of suffering, this is certainly no reason to accept a culture of ignorance.

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