Birds of a **feather**

Using less traditional avian models in laboratory research means **Dr Charlotte Cornil** is more easily able to study distribution and regulation of brain aromatase and its interaction with other neurochemicals

How did you become interested in the mechanisms that control sexual differentiation and activation of reproductive behaviours?

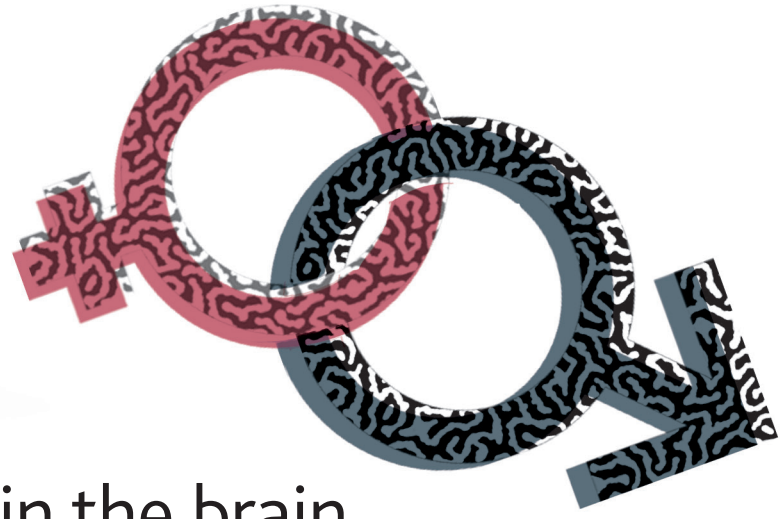
It all started with an encounter with Dr Jacques Balthazart, who was well known for his work on the behaviour of Japanese quail. I was stunned by the effect of oestrogens on sexual differentiation of brain and behaviour – a single injection of oestrogens into a genetically male egg is sufficient to feminise the behaviour of the adult. Subsequently, I was given the opportunity to work on the relatively new idea that oestrogens could act much more rapidly than anticipated. This completely changed the way I think about hormone action. I find the diversity and power of the hormones fascinating; they literally control every biological system, from the immune response to the brain.

Can you briefly outline the roles of the hormones on which your research focuses?

Testosterone and oestrogens are commonly referred to as male and female hormones. Indeed, each circulates at higher concentration in the blood of the sex they are characteristic of, and are mostly known for their action on functions typical of males and females respectively, such as the development of secondary sexual characteristics or reproductive functions. Yet, they have many more important roles. For example, in both sexes oestrogens affect processes such as bone and tumour growth, sensory perception, learning and memory. Importantly, oestrogens are produced from androgens. This conversion – called aromatisation – mainly takes place in the ovary but also occurs in other tissues including the brain. It plays a key role for the masculinisation of the brain and the activation of sexual behaviour in males of numerous species.

Why are avian models particularly appropriate for your work?

Avian species offer several advantages for the questions we investigate in our laboratory; one of which is the higher concentration of brain aromatase compared to mammals. The dual regulation of aromatase by genomic and non-genomic mechanisms was first demonstrated in quail. Japanese quail are also easy to breed, readily display behaviour in captive conditions and their behaviour is tightly controlled by steroid hormones, making them a good model for studying the underlying mechanisms of hormonal action. Of course, there are downsides to the study of 'exotic' species such as quail or songbirds. These are mainly technological, as the study tools are not as developed as those for more common species such as rats or mice. For example, antibodies to detect mammalian proteins do not always recognise their avian counterparts. Similarly, the quail genome has not been sequenced, which renders investigations more complex.



Sex in the brain

Work underway at the University of Liège is contributing to the body of knowledge about the ways in which brain oestrogen production is modulated by social and environmental changes that can rapidly alter behaviour

What challenges have you had to overcome to make progress in this field of research?

The fast actions of oestrogens had mostly been studied at the cellular level, such that little was known about the functional implications of associated events for the whole organism. The high cooperation of the fast and slow modes of action was the first issue that had to be dealt with as we needed to find the most appropriate hormonal conditions in which to study this question. It took us several years of playing with endocrine treatments before we found a good approach. This resulted in a series of robust results showing that acute changes in brain oestrogen concentration rapidly and specifically control sexual motivation, while sexual performance strictly depends on their classical mode of action via changes in genomic transcription. Along with the acute enzymatic changes observed both *in vitro* and *in vivo*, these results prompted us to propose that neuroestrogens could be considered as neurotransmitters, or at least as neuromodulators.

How does your research benefit from collaborations with scientists external to your group?

Long-lasting collaborations involve discussion of research lines, grant applications and experimental designs as well as student exchange. Yet more involve joining forces to answer a common question. Our field of research is pretty friendly, and sharing methodological protocols or tools such as antibodies with other researchers is common practice.

OESTROGENS ARE A group of steroid hormones most commonly recognised as the primary female sex hormone, important for regulating the menstrual cycle and pregnancy. These compounds, however, have many more wide-ranging biological functions, including sperm production, cardiovascular protection, immune response, neuroprotection, bone remodelling and control of sexual behaviour.

An understanding of the different cellular mechanisms underlying the actions of oestrogens has been in place for a number of years. The ways in which these translate into physiological and behavioural control, however, has continued to evade scientists. The functions of oestrogens are mediated through the activation of their receptors, which are expressed in target cells. The mechanisms by which cells respond to oestrogens are highly complex and poorly understood. This is in part because of the functionally distinct nature of the two receptors, which have different tissue distributions, interact with different signalling proteins and thus play different – often opposing – roles in gene activation.

Research being carried out by Dr Charlotte Cornil and her team at the University of Liège, Belgium, is striving to unlock some of these secrets, in particular, the way in which oestrogens differentially impact on the brains of males and females. Reaching beyond the realms of neuroendocrinology, this work delves into identifying the neuroendocrine and neurochemical mechanisms controlling the sexual differentiation and activation of reproductive behaviours, including the interaction between steroids and neurotransmitters. They are working on the foundation that expression of aromatase – the enzyme that converts the androgen testosterone into the oestrogen oestradiol – in the brain can influence sexual behaviour.

A pair of Japanese quail mating.

THE ROLE OF NEUROESTROGENS

A growing body of evidence demonstrates that oestrogens activate a large variety of cellular signalling pathways through membrane-initiated events that are too rapid to result from *de novo* protein synthesis. Cornil's group is well positioned to understand more about oestrogens' impacts at the organism level as they have developed the necessary skills and expertise to utilise a range of methods and technologies in their investigations, including behaviour analysis, enzymatic assays, radioimmunoassays and molecular biology. Cornil has used a novel and less conventional experimental model to explore the genomic and non-genomic influence of neuroestrogens – oestrogens produced by the brain – on male sexual behaviour.

Japanese quail were used to study the ways in which central administration of oestradiol facilitates male sexual motivation. To do this they deprived males of oestrogens through treatment with an aromatase inhibitor that resulted in an acute impairment of sexual motivation but not performance. Oestradiol or a membrane-impermeant oestradiol analog restored this sexual motivation within minutes. "These results indicate that complementary mechanisms have evolved, allowing oestrogens

INTELLIGENCE

DUAL ACTION OF NEUROESTROGENS ON THE REGULATION OF BEHAVIOUR

OBJECTIVE

To gain insights into the mechanisms by which oestrogens acutely impact brain physiology and behaviour by studying the brain circuits controlling sexual behaviour in both males and females.

KEY COLLABORATORS

Dr Gregory F Ball, University of Maryland, USA • **Dr Kevin S Holloway**, Vassar College, USA • **Dr Colin J Saldanha**, American University, USA • **Dr Christina Dalla**, University of Athens, Greece • **Dr Thierry D Charlier**, Université de Rennes, France • **Dr Annemie Van Der Linden**, Universiteit Antwerpen, Belgium • **Dr Ilse Smolders**, Vrije Universiteit Brussel, Belgium • **Dr Jean-François Arnal**; **Dr Françoise Lenfant**, Université de Toulouse, France

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CHARLOTTE CORNIL was awarded her Master's in Biology and PhD from the University of Liège, Belgium, before moving to Johns Hopkins University, USA, for a postdoctoral research position. Following this she returned to Belgium, first as a postgraduate researcher, before rising to Research Associate from the FRS-FNRS in 2009. Cornil has been awarded numerous prizes and travel awards in recognition of the success of her research to date.

Université de Liège

to act at the cell membrane in different time frames, control distinct components of the same behavioural response and improve reproductive fitness," Cornil elucidates.

Cornil hopes that her work will provide new insights into the drivers of sexual behaviour: "The development of drugs specifically targeting membrane oestrogen receptors may thus lead to significant therapeutic applications". The observation that males treated with an aromatase inhibitor suffered from reduced sex drive has implications for drug development: "Treatment with aromatase inhibitors or anti-oestrogens in the context of chemotherapy, for example, may have dramatic effects on the life quality of patients – highlighting the potential impact of this work," she postulates.

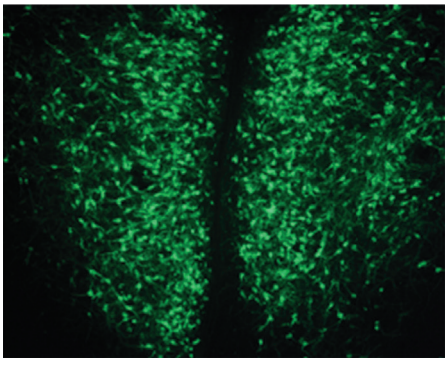
STEROID METABOLISM

Genomic and non-genomic factors also regulate aromatase activity at varying spatial and temporal scales. The genomic control of aromatase concentration, via the transcription rate of the corresponding gene, is a relatively slow process and changes are only observed after a couple of days, with the ultimate impact on behaviour detected after one to two weeks. In contrast, non-genomic control affects the efficiency of the enzyme to convert testosterone into oestradiol, the effects of which can be observed within just a few minutes.

A challenging component of their inquiries has been to establish the anatomical site where rapid changes in aromatase activity takes place. This is because the concentration of both oestrogens and aromatase in the micro-dissected samples is extremely low. The researchers have been able to show that there are changes in aromatase activity immediately after sexual interaction in specific regions of the brain, depending on the sex of the individual and the stimulus: "Our observations indicate that the regulation of local provision of oestrogens in the brain is complex and neuroestrogens are potentially involved in the control of many neurophysiological processes," Cornil explains. The group has now begun to characterise a transgenic mouse model that is carrying a mutated oestrogen receptor. This prevents trafficking to and signalling from the cell membrane. The aim of this research is to provide additional evidence to support the hypothesis that genomic and non-genomic effects of oestrogens interact to control reproductive behaviour over both the long and short term.

OESTROGENS AS NEUROMODULATORS

In other *in vitro* experiments, the team found that the acute regulation of aromatase activity was operated by post-translational modifications of the enzymatic protein, such as addition of a phosphate group (a cellular process called phosphorylation). They then demonstrated that changes in the social or environmental context impact the enzymatic activity of brain aromatase. "These sex-, brain region- and stimulus-specific enzymatic fluctuations provide a mechanism of acute



Aromatase immunoreactive cells in quail preoptic area.

regulation of local oestrogen provision with a time and spatial resolution that fits with the rapid effects of oestrogens observed on male sexual behaviour," points out Cornil. Their work noted that the response seemed to be reliant upon the female being present so is likely to be linked to male motivation.

While it is recognised that changes in ovarian secretion control reproduction, the fact that the female brain abundantly expresses aromatase is something that holds much interest for Cornil. The finding that female brain aromatase activity is also acutely altered by environmental changes inspired the team to begin investigating the role of neuroestrogens in females. For example, depression is known to be more prevalent in women in the reproductive stage of their life and it is suspected that oestrogen levels are a contributing factor. What is particularly interesting is that when the results of their experiments are analysed together, there is evidence that brain-derived oestrogens can be viewed as neuromodulators. "Our work suggests there are two distinct mechanisms of action of oestrogens acting in different time frames to control different components – motivation and performance – of the same behavioural response and improve reproductive fitness," expounds Cornil. Excitingly, since aromatase plays a key role in controlling steroid-dependent processes, there is much potential for their findings to extend to other systems.

A NEW MODEL

The Cornil group's efforts have provided a novel model integrating two different modes of oestrogen action. These control the same behaviour but via distinct temporal domains. Since oestrogens produce more than one effect, it is likely that the results from this study can extend to other behavioural responses that are associated with reproduction, such as parenting, as well as non-reproductive responses, such as cognition – therefore providing some valuable information for other research. Their work highlights the importance of taking both sexes into account when investigating drug efficacy. This is because there can be major differences in how a drug influences the functioning of the brain and body, which can significantly affect how susceptible someone is to a disease and how it progresses; all of which is valuable information for the development of new treatments.