The game of smells

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At the end of 2008, the magazine *Scientific American* made a point of mentioning a discovery made by researchers at the ULg's GIGA neurosciences unit, published in the journal *Behavioural Brain Research*. The Liège neuroscientists had just brought to light the involvement of certain olfactory stimuli in the reproductive behaviour of the Japanese quail. Confirmation that these birds were able to perceive smells despite having a poorly developed olfactory bulb.

That vultures more often locate their prey thanks to a highly developed olfactory sense has never been contested. Different experiments have moreover shown that such was the case that visual clues don't play a predominant role in guiding them to decomposing animal carcasses. Around forty years ago, Bernice Wenzel, of the University of California at Los Angeles, became interested in other birds in a similar framework. She highlighted the fact that it was also through a sense of smell that the...
kiwi, the flightless New Zealand bird equipped with rudimentary wings, detects its food, buried in the sand. In the same way she also demonstrated that various sea birds, such as petrels, go on smells given off by dead fish to fly back against the wind to the shoals from which they draw their food. A simple but instructive experiment consisted of placing a sponge soaked in cod liver oil on an offshore raft. The sea birds flocked towards her, against the wind (figure below).

Up until around the beginning of the 1970s, scientific studies in their entirety considered that all land birds, beyond vultures and kiwis, lacked a sense of smell. Operant conditioning techniques were thus used by certain laboratories to revisit the question. From this work it emerged for example that, contrary to common opinion, pigeons perfectly discriminate smells, at least certain amongst them, but we still don’t know to what exact ends they use this faculty.

However the work by Floriano Papi and his colleagues at the University of Pisa provided a cluster of convincing clues according to which carrier pigeons draw up an 'olfactory map' of the region in which they live and could make use of it to get their bearings. As is underlined by professor Jacques Balthazart, of the GIGA Neurosciences (formerly the University of Liège's Centre of Cellular and Molecular Neurobiology) the birds obviously do not smell their pigeon loft from a long distance but, according to the hypothesis, could connect the smell of the sea or the smell of a forest to wind direction: 'Whilst it is contested by certain people, this theory has been confirmed in independent ways by many laboratories,' he adds.

A question of size...

Already by the 1970s, Jacques Balthazart had shown that cutting the olfactory nerve inhibited courtship displays and sexual behaviour in ducks, which suggested that, much as for rats and other rodents, for whom
smells allow them to distinguish between a male and a female or between a female which is sexually receptive and one which isn't, ducks use olfactory signals in their 'private life.'

A burning question on many people's lips: having being established for several decades, didn't the presence of the olfactory nerve in animals allow it to be supposed, before 1970, that they were equipped with a sense of smell? Here, everything is a question of nuance, or more exactly of size. In the 1960s scientific work had concluded that quite normal olfactory structures existed in birds: their nasal cavity well and truly possessed a mucosa (mucous membrane) connected by a nerve to an olfactory bulb, itself connected to the rest of the brain but according to a schema which is poorly understood. Anatomists had underlined the great disparity in the size of the olfactory bulbs according to species: very small for the sparrow, but enormous in relation to the size of the brain for the petrel or the vulture. And from there sprang the conclusion, stated at the time and which prevailed for decades: birds which had small olfactory bulbs - the most frequent case beyond some exceptions such as vultures, petrels or kiwis - cannot smell anything, or as good as.

Different researchers have shattered this dogma into pieces over the last five years. Studies of the behaviour of the tit illustrate this very well. What has been observed? That they mark a pause before going into their nests and that they stay there for less time than usual if one has scattered the odour of one of its predators, such as the stone marten, around it. At the same time, we know that the Corsican tit gathers aromatic plants into its nest in order to keep parasites at bay. If they are removed against its will it hesitates a moment before going inside, which suggests that it has picked up on a change in its environment, without having seen it.

The scent of the female

It is for over thirty years now that professor Balthazart has been interested in the neuro-anatomical bases by which the complex behaviours of the higher vertebrates are controlled. He approaches this problematic by looking at the reproductive behaviour of the Japanese quail (*Coturnix japonica*) and also, since a few years ago, that of mice. In October 2008, the magazine *Behavioural Brain Research* published an article of which he is the co-author, called Site-specific effects of anosmia and cloacal gland anesthesia on Fos expression induced in male quail brain by sexual behavior(1). The Ariadne's thread of this contribution is a question which
leads us back to the problematic of olfaction in birds: does manipulating the sense of smell of the Japanese quail, if the latter can be said to have one, produce effects on the animal's reproductive behaviour? Jacques Balthazart and his team were not starting from a blank sheet of paper in broaching this subject. In fact certain data gathered earlier had suggested, without demonstrating it, a link between sexual behaviour and olfaction in the quail.

We have known for about twenty years that most nerve actions have as a correlate an increase in the synthesis of a protein, named Fos. Thus if you submit a quail plunged into darkness to flashes of light, you will observe a heightened synthesis of the RNA messenger for the Fos protein in its visual system 15 to 20 minutes after the administration of the stimuli and of the Fos protein itself 60 to 90 minutes after the same stimulation. If you cover up one of the animal's eyes, this phenomenon will only take place on one side of the brain. And if the quail has an epileptic crisis the Fos protein will make its presence felt in the affected cerebral area. A few years ago several experiments carried out at Liège brought to light another element which is inscribed in the same logic: when the male quail mates and one takes a sample of its brain 90 minutes later, we can see Fos appear in very precise cerebral zones, including the median preoptic area and the bed nucleus of the stria terminalis, two regions involved in sexual control and activities of an analogous nature in rats in the case of copulation. However, in the rodent the induction of Fos in these circumstances is linked to the perception of the smell of the female and to sensorial stimuli from the penis. 'If you anaesthetise a rat's nostrils it will copulate less and when it does so it will synthesise Fos a lot less,' reports professor Balthazart. 'The same will happen if you cut the nerves in the genital area. And if you carry out the two operations it will produce really small amounts of Fos.' That suggests that activation of the protein doesn't depend on the motor execution of sexual behaviour but on the perception of olfactory stimuli emitted by the female and genital stimuli originating from the penis.

(1) Mélanie Taziaux, Matthieu Keller, Gregory F. Ball and Jacques Balthazart, Site-specific effects of anosmia and cloacal gland anesthesia on Fos expression induced in male quail brain by sexual behavior, in Behavioural Brain Research, 194 (2008), 52-65.

An X rated film

This could not be of greater interest to the ULg researchers. All the more so because on the one hand the quail was not a priori supposed to perceive smells and on the other because birds, with the exception of ducks and ratites, are not equipped with a penis - copulation takes place through apposition of the cloaca, an orifice common to the intestinal, urinary and genital tracts. What was happening then? That is what the GIGA neurosciences team, under the management of professor Balthazart, set out to discover.
A first experiment consisted of measuring the production of Fos and to observe the behaviour of males who had either had their nostrils blocked in order to prevent olfactory stimuli, or whose cloacal region had been anaesthetised in order to switch off genital stimuli, or who had been submitted to both operations. Two other populations were taken into consideration: control animals which stayed in their cages without seeing a female and others who saw a female and copulated but whose sense of smell and genital areas had not been manipulated. The animals of the first three groups were each placed in the presence of a female. At the level of behaviour there was no difference between them and their 'un-manipulated' counterparts: they copulated as if nothing had taken place. 'It is not because neither the blocking of olfactory stimuli nor the sensitiveness of the cloaca didn't affect the birds' behaviour in a noticeable fashion that nothing had happened,' says Jacques Balthazart. 'Nonetheless all the measurements that we carried out revealed that the time that had passed between the introduction of a female and the beginning of sexual behaviour conformed to what occurs normally.'

He nonetheless adds that to be really sure that sexual activity including cloacal juxtaposition and the transfer of sperm had occurred it would be necessary to use high speed cinematography. Up until know this 'X rated film' has not been shot...It would also be interesting to study the fertilization rates of the eggs laid by the females after copulation. If they turn out to be lower it would have to be concluded that a modification in the effectiveness of sperm transfer had occurred.
The same as for rats?

And the Fos protein? Here we have a change of scenery. The activation of the brain linked to copulation was partially lost in the three groups which had been manipulated. The phenomenon was limited to the median preoptic area and the bed nucleus of the stria terminalis and didn't affect other areas, such as the amygdala, considered to be the centre of emotions. This structure was activated in a non differentiated way in all the groups which had copulated, including the group containing the individuals whose noses had been blocked and whose cloaca had been anaesthetised.

In submitting the immunocytochemical staining of the Fos protein to quantitative microscopic analysis, it is possible to evaluate the number of cells which express the Fos gene in various areas of the brain. They number around twenty per histological section of the bed nucleus of the stria terminalis in the control animals. If the latter copulate with a female the number rises to more or less forty-five when a count is made ninety minutes after mating. In the case of the cloacal area having been anaesthetised it is around twenty in the same conditions and in the case of anosmia or the blocking of both olfactory and genital stimuli, around twenty-five to thirty. A similar phenomenon occurs in the median preoptic area.
In themselves the results are not very surprising if we refer to studies which focus on the rat. In effect, let us remind ourselves, it has been demonstrated that it is not the sexual activity itself, but the olfactory and genital perceptions which lead to the production of the Fos protein in rats. What is surprising is that the induction of Fos should be affected by the manipulation of the olfactory system in a bird which is not supposed to use olfaction in its sexual life. Can comparisons be misleading? The question has been put, as has been the following one: do quail continue to copulate in the absence of stimuli judged to be potentially important for the beginnings of sexual behaviour to take place? Here we need to make something clear: the birds used in the work by the GIGA Neurosciences researchers were sexually experienced. This is an essential element if we want to continue making comparisons with the rat. In fact in the latter a male without sexual experience bases itself on an ensemble of olfactory, visual and acoustic information to identify a female as such and to determine if she is receptive. Later just one type of perception will be sufficient.
According to Jacques Balthazart a similar phenomenon can be envisaged in the quail. But with one different nuance, for in birds sight is predominant - they can undertake nothing if they are deprived of it. Earlier experiments by the Liège team have moreover stressed that a male quail in the habit of copulating does not hesitate a second before mating with a stuffed bird. It is thus possible that an inexperienced bird must lean on information springing from various sensory channels before getting on with the sexual act and that afterwards just the the sight of a female is sufficient. 'Smell would thus seem to be a discriminatory stimulus during the animal's first sexual contacts and then becomes simply a reinforcing stimulus,' points out our contact. A hypothesis that he will look into during his next experiments.

One thing is nonetheless certain: the work published in Behavioural Brain Research confirms that so called microsmic birds, in other words birds which possess very small olfactory bulbs, receive olfactory stimuli which activate brain cells at the very depths of their brains. A sign of the quality of the GIGA Neurosciences scientific research is that their experiment was recounted on the 24th of November in the very well known magazine, Scientific American. An article entitled The Scent of a Warbler: Birds May Use Sense of Smell in Mating(2).

In September 2008, Jacques Balthazart and Mélanie Taziaux, a doctoral student at the au CNCM, had in addition published a review of the literature related to olfaction in birds and its role in reproductive behaviour. The title of this contribution, also published in Behavioural Brain Research: The underestimated role of olfaction in avian reproduction?(3)

(2) Adam Marcus, The Scent of a Warbler : Birds May Use Sense of Smell in Mating, in Scientific American, 24 novembre 2008. Read the article


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