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- Cats' preference for pyrophosphates and search for a feline taste receptor using molecular biology
- How does feeding behavior develop in domestic cats

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Cats' preference for pyrophosphates and search for a feline taste receptor using molecular biology

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Pyrophosphates (PP) are compounds with a P2O7 anion (fig.1). They are available as di-, tri- and tetra-sodium salts ($Na_2H_2P_2O_7$, $Na_3HP_2O_7$ and $Na_4P_2O_7$, respectively), as well as several non-sodium forms. They have numerous common uses, among which we can find petfood applications such as palatability improvement in catfood or urinary pH regulation.

SPF includes inorganic phosphate compounds in some palatability enhancers (cf patent applications : WO 2003/039267 and WO 2007/118876). So far, the way of action of PP is not totally understood but this study demonstrates the possibility that pyrophosphate could act as a modulator of the activity of the cat's amino acid receptor (T1R1/T1R3).

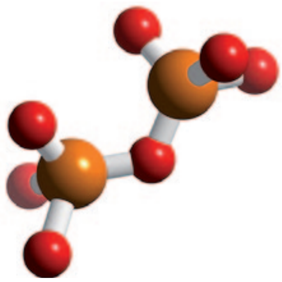

Figure 1 : Pyrophoshate molecule (P2O7 4-)

CAT'S TASTE HAS EVOLVED IN A SPECIFIC WAY

Each taste modality (sweet, umami, bitter, sour and salty) has unique receptors. The taste receptors for sweet and umami share some characteristics. For example, they are coupled to a regulatory "G" protein, for generation of intracellular second messengers, making it a G protein coupled receptor (GPCR). Convention now holds that the receptors of this family are called "T1R" receptors (Taste Receptor of class 1 structure).

Ex :
- The dimer T1R1/T1R3 recognizes amino acids (umami sensation in humans)
- The dimer T1R2/T1R3 responds to sweet stimuli

Cats are obligate carnivores : they mainly eat flesh and they have even lost the ability to detect sweet taste through the taste receptors (T1R2/T1R3) used by other species, including dogs. This lack of sensitivity is due to a genetic mutation : in the cat, the T1R2 receptor has been found to be an unexpressed pseudogene, thus leading to the impossibility of having a functional sweet taste receptor (Li et al, 2005). The recent discoveries related to the cats sweet taste receptor provides an example of how a gene can be linked to behavior and also alter the metabolism of nutrients. Cat's feeding behavior is mainly driven by its sensitivity to amino acids. The dietary consumption relies on major appetitive receptors, T1R1/T1R3, inducing the specific umami taste. When several amino acids seem sweet to us, they are perceived differently by the cat as they interact with the T1R1/T1R3 receptors. The Monell Chemical Senses Center has already identified the coding sequences of cat taste receptor subunits T1R1 and T1R3, using reverse transcription/polymerase chain reaction (RT/ PCR) of cDNA obtained from cat vallate and clavate papillae (patent application WO 2005/005480).

HOW DO PYROPHOSPHATES WORK : A HYPOTHESIS

One possible mechanism of action of how pyrophosphates enhance palatability of cat foods is a direct taste enhancing effect, generated by an interaction with specific taste receptors.
There is a very good synergy between phosphate salts and meat hydrolysates : the result obtained by the association « PP + meat hydrolysates » is significatively better than with meat hydrolysates alone. Recent palatability trials have shown that pyrophosphates act best when mixed with hydrolysates containing free amino acids (patent application pending). We therefore hypothesized then that the taste receptor (T1R1/T1R3), which recognizes amino acids, could be involved in the recognition of PP in cats.
Thus, the aim of this study, conducted in collaboration with Monell Chemical Senses Center, was to investigate the effect of trisodium pyrophosphate on feline taste receptor T1R1/T1R3.

WAYS OF INVESTIGATION

The molecular and biochemical study has been based on the heterologous expression of the umami feline taste receptor (T1R1/T1R3) transferred into human embryonic kidney cell line (HEK 293). This cell line was transfected with the polynucleotides encoding the feline T1R1/T1R3 receptor and the G protein, G-α15.

The cells containing T1R1/T1R3 receptors were loaded with fura-2, a sensitive calcium indicator dye that measures intracellular calcium concentration. We measured the responses of these cells to stimulation using fluorescence ratiometric determination of intracellular calcium.
When a receptor was activated, an increased calcium activity was indicated by a dye signal. The activation of the receptors was measured by the visualisation of the calcium flux, in the presence of difference tasty solutions (amino acids, PP alone or association of both).
The cells expressed a robust response to isoproterenol, used as a positive control, showing the reliability of the method.

RESULTS

Different stimuli, such as amino acids and trisodium pyrophosphate, were tested. The expressed feline T1R1/T1R3 receptors showed to be sensitive to various amino acids but they were also stimulated by solutions containing pyrophosphates.
The enhancement of the response recorded depended on the nature of the amino acid and the concentration of the compounds tested.
Several cells displayed little activation to an amino acid and/or to PP alone but the combination of some amino acids) with PP showed a high degree of enhancement, suggesting a hyperadditivity mechanism or synergism ; i.e. : a Lys+PP mix was able to induce a more important activation than the arithmetic sum of the responses to the single compounds. Similar results were obtained for L-alanine and L-proline mixed with PP.

More cells responded to the mixture of PP plus amino acid than to PP alone or to any amino acid tested, suggesting recruitment of cells that were weakly or non-responsive to either of the compounds alone (fig.2 & 3).

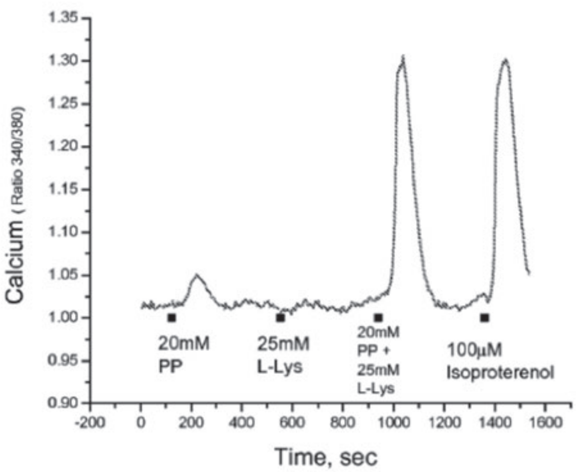

Figure 2 : HEK293 cell expressing feline T1R1/T1R3 receptor stimulated by a mixture of trisodium pyrophosphate and L-Lysine

HEK293 cell expressing feline T1R1/T1R3 receptor and Gα15 gave a weak response to 20mM trisodium pyrophosphate and a null response to 25mM L-lysine alone. However, there was an enhanced response to the mixture of 20mM trisodium pyrophosphate and 25mM L-lysine. 100 µM isoproterenol serves as positive control.

HEK293 cell expressing T1R1-T1R3 receptor and Gα15 gave null responses to 50mM L-proline, 10mM and 20mM trisodium pyrophosphate presented singly but a robust response to the mixture of two compounds 20mM of trisodium pyrophosphate and 50mM of L-proline. 100 µM isoproterenol serves as positive control.

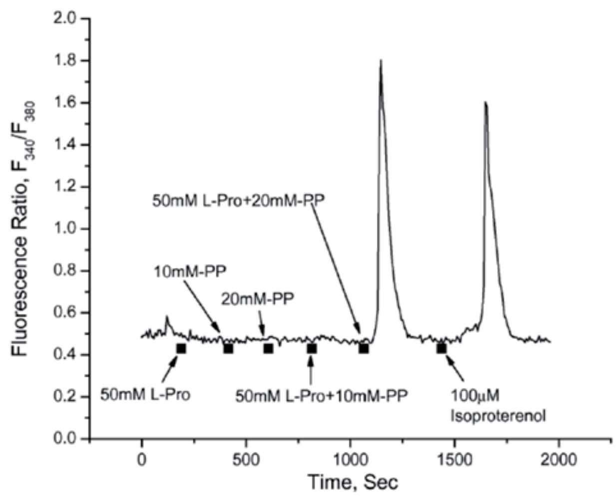

Figure 3 : HEK293 cell expressing feline T1R1/T1R3 receptor stimulated by a mixture of trisodium pyrophosphate and L-Proline

CONCLUSION

The developed method can allow the screening of different compounds and the determination of their potential as cat palatability agents. This method has been patented (WO 2012/013480) : it allows determining if two or more test compounds have an augmenting effect on the T1R1/T1R3 receptor where each test compound elicits a small or moderate response.

How does feeding behavior develop in domestic cats

Claire Larose Forges, Aurélie Becques, Julien Rogues, SPF, Elven, France.

MOTHER'S FEEDING & KITTEN'S PREFERENCE

Cat preferences for food can be inherited and may be linked to the mother's feeding during gestation and lactation. SPF in collaboration with Paris13 University, performed an experimental study to investigate the relation between the mother's feeding and the kitten's preference (Becques, 2009). The protocol (fig.1) was based on the comparison of two groups of 7 pregnant females.

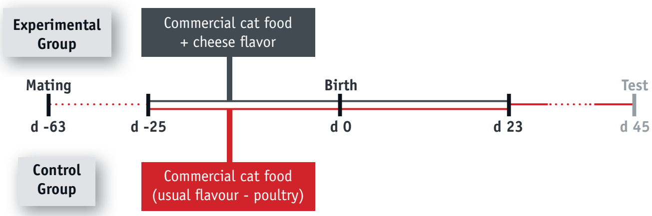

Figure 1 : Protocol

The "control group" was exclusively fed with a cat food + usual poultry flavor before and after birth of their kittens. The "experimental group" was fed with a cat food + cheese flavor unknown to the cats. Two groups of 15 kittens were evaluated in term of flavor preferences at 2 days or 45 days after birth. For the 2-day-old kittens, we observed their behavior when individually placed in a small box equipped with two flavor-soaked cotton balls 1 with cheese flavor and 1 with poultry flavor (fig.2). All kittens exposed to cheese flavor via maternal diet during pregnancy crawled to the cheese flavor cotton. The 45-day-old observation was based on first choices and intake ratios (fig.3). A two-bowl test was performed comparing small chicken meat pieces sprayed with cheese flavor vs chicken flavor. All kittens exposed to cheese flavor via maternal diet during pregnancy and weaning chose first and ate significantly more of the bowl with cheese flavor. These results show that antenatal, perinatal and postnatal exposure significantly influences olfactory preferences in kittens from birth till weaning.

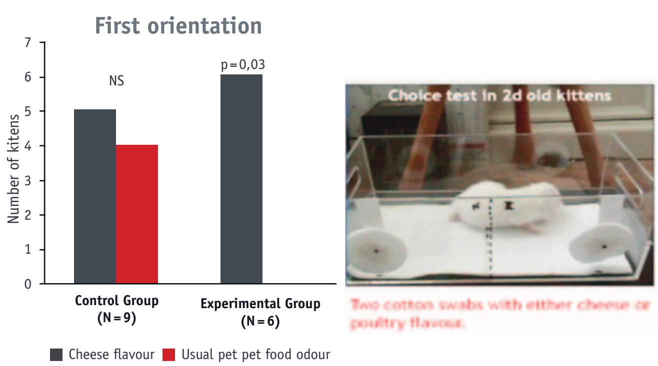

Figure 2 : Results of 2-day-old observation

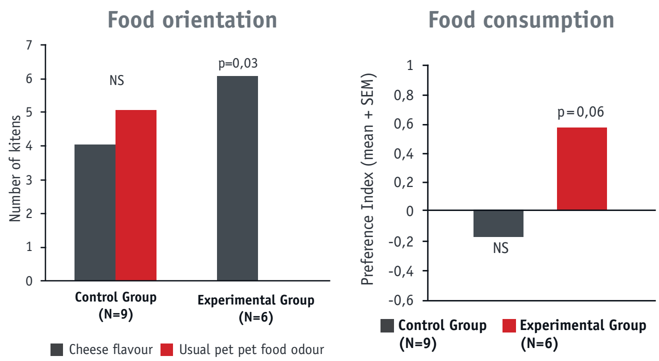

Figure 3 : Results of 45-day-old observation

INFLUENCE OF BEHAVIORAL & SOCIAL ELEMENTS

Over time, the food preferences of cats can be also acquired. Depending on their dietary past, their tastes evolve. Kitten preferences are influenced by behavioral and social elements. At weaning, young cats are fond of the food their mother likes to eat (Wolter, 1982). Cats reared from weaning to two years old on specific diets will develop neophobia reaction towards unknown food and will express strong preference for their usual food (Kuo, 1967, Brashaw, 2006).

Inversely, cats which had previously experienced a variety of diets will develop neophilia reaction towards novel diets and will express an instantaneous preference for them (Mugford, 1977, Thorne, 1982). With a good palatability level and repeated exposure, the first profile of cats may accept small quantities and get accustomed to a new food. The second profile of cats may rapidly express a preference truly related to palatability and not only to novelty effect. Finally, even though variation of diets is important from weaning, different expression of individual feeding behavior can exist. Some cats develop strong preference for the usual diet (Bradshaw, 2000) or weariness towards it, inducing palatability decrease (O'Malley, 1998).

LEVEL OF EXPOSURE & CAT'S PREFERENCE

To better understand which parameters could explain this last contradiction, data from PANELIS— the expert center dedicated to palatability measurement and feeding behavior study, was used in an extensive statistical analysis. PANELIS performs versus palatability tests with over 385 cats, fed with different dry diets on a daily basis. All cat characteristics (age, sex…) and product specificities were taken into account. The aim of this analysis was to study the relation between the level of exposure to a food feature and the cats' preference for foods exhibiting this feature.
From 2007 to 2010, 350 000 individual data points were collected at PANELIS. This palatability test data was organized by criteria such as the type of coating fat, the nature and manufacturing process of palatability enhancers (PE). These characteristics trace the exact feeding experience of each cat. The degree of exposure was then statistically processed using two forms of multivariate data analysis : PCA (principal components analysis) and HCPC (hierarchical clustering on principal components).

• The first set of analysis establishes that these cats can be grouped in classes based on their feeding history. Figure 4 (PCA analysis) highlights the contrast between cats with a high or low level of exposure to all features of the palatability enhancers. Figure 1 shows that cats at the top of the PCA chart were also more exposed to products without pyrophosphate whereas cats at the bottom of the chart were more exposed to a combination of liquid and powder palatability enhancers.
• The second level of analysis suggests a link between the feeding history and the development of individual preferences.

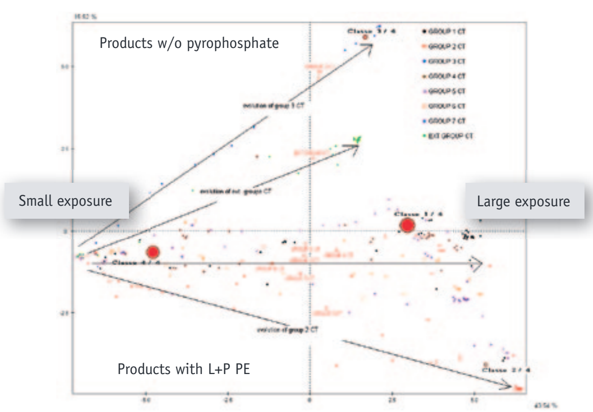

Figure 4 : PCA plot of explosure level for cats and catteries
Cats were grouped into 4 classes of exposure (yellow circle)

As an example, figure 5 highlights the evolution of preferences in relation to the level of exposure to the feature known as "biochemical reaction level 2" (BioR 2). The preference for BioR 2 is impacted positively by that feature repeated exposure.

Distribution of BioR 2 Individual preferences depending on the level of exposure to BioR 2

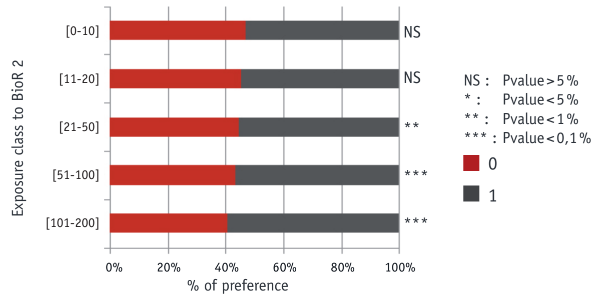

Figure 5 : Results for the biochemical reaction 2
Preference to food presenting the BioR 2 feature increases through repeated exposure.

CONCLUSION

These results confirm that feeding preferences change over time and are influenced by a lifetime of feeding experiences. Depending on the products' features, the effect of the dietary past of a cat can be varied as such :

- **the preference for a product is strengthened through repeated consumption**
- **the dislike for a product is developed through repeated consumption**
- **the preference for a product is acquired right from the first consumption and remains stable through repeated consumption**

RELIABILITY OF RESULTS

Therefore, the study demonstrates the importance of recording and controlling the feeding experience of cats involved in palatability studies. To avoid potential bias due to past feeding history, PANELIS has developed a strong expertise in animal feeding behavior, cattery management and data analysis. From their recruitment, the kittens are trained on a diversified diet to avoid feeding imprinting. Once adults, when they start to perform palatability tests, PANELIS takes continuous care in preserving food diversity. Additionally, trials are organized and controlled to avoid unbalanced diet experience between cats. Comparative sessions of tests with external panels are also regularly organized. This standardization process is strategic to maintaining relevance and reliability of results.

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CATS' PREFERENCE FOR PYROPHOSPHATES AND SEARCH FOR A FELINE TASTE RECEPTOR USING MOLECULAR BIOLOGY

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HOW DOES FEEDING BEHAVIOR DEVELOP IN DOMESTIC CATS

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