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Taste and Smell: from Molecular Biology to Behaviour

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Philip James (moderator): We have had some fascinating presentations ranging from the detailed molecular analyses of olfactory and taste systems through assessments of integrative pathways and how the brain adapts and responds in behavioral terms to the array of olfactory and taste inputs.

Dr. Ryba has given us an illuminating account of the nature of the taste receptors [Editor's Note: The summary of this presentation was not available for publication here]. He told us that the sense of taste depends on a family of three specific taste receptors, the T1Rs, which are G-linked receptors with long extracellular domains. There are subsets of cells within the taste buds which express individual T1Rs. Thus, T1R1 is most prominent at the front of the tongue in the fungiform taste buds and in the palate, whereas T1R2 receptors are more prominently expressed towards the back of the tongue in the foliate and circumvallate papillae. The T1R3 receptor, however, has a much more general distribution in all the different types of taste bud from different regions of the tongue and palate, so he was able to define three classes of taste receptor cells: cells that express T1R1+3, cells that express T1R2+3, and cells that express T1R3 alone.

Further fascinating experiments were described, which revealed the structural differences between the TIRs of different species and which defined the mouse or human's capacity to respond to and therefore discriminate between different tastes. Often, the appreciation of different tastants depended on the coexistence of two or more genetic forms of receptor in each taste receptor cell. The discrimination of many forms of sweet tastants, including both natural and artificial sweeteners, depends on a combination of T1R2+3. The responses to L-amino acids and the distinctive flavors found in meat—the umami sensation—are mediated by the activation of T1R1+3 receptors. In humans the umami receptor selectively responded to glutamate alone, whereas rodents are more broadly tuned through their molecular forms of T1R1+3 to respond to many other amino acids. He then set out how the bitter taste is mediated by the activation of a population of cells which express up to 30 different forms of the T2R receptors.

Given these astonishing developments in our molecular understanding of taste, perhaps we should start the discussion on this issue.

Christian Drevon: If we consider taste receptors as very important, do we know of any mutations in recep-

tors in humans, and, if so, how does this alter the signaling system with perhaps physiological or indeed even pathological consequences? The next question is, are there any racial differences? Surely such a simple issue as sweet taste could be used to discriminate receptor responses to sweetness in Japan compared with, for example, the Middle East? The diets are completely different: is this biologically entrained?

Nick Ryba: I have discussed already how the sense of taste depends on a family of specific taste receptors which show structural differences between species and which define their capacity to respond to and therefore discriminate between tastants. Different receptor types are distributed in different parts of the tongue and palate and the appreciation of different tastants depends on the coexistence of two or more genetic forms of receptor in each taste receptor cell. Mice and humans differ in responding to selective tastes because of the sequence differences in the receptor genes as shown by knockout and transfection experiments. The umami sensation is mediated by the activation of T1R1+3 receptors, while the bitter taste is mediated by the activation of a population of cells which express up to 30 different forms of the T2R receptors.

The Distribution of Different Taste Receptors in the Tongue

Nick Ryba: I will start by highlighting a small family of three taste receptors, the T1Rs, which have, in molecular terms, seven transmembrane helices characteristic of G-protein-linked receptors. They are also characterized by having long extracellular domains. The T1Rs are a family of G-protein-linked receptors which are related to other forms of sensory receptor, e.g. the calcium sensing receptor and a large family of mammalian pheromone receptors. They are expressed in taste buds, and, by assessing their distribution within taste buds with in situ hybridization techniques, it becomes evident that subsets of cells within the taste buds express individual T1Rs. This is the pattern of expression that we would expect for sensory receptors involved in discriminating between different tastes.

We found that there are different topographical distributions of the three T1Rs in the taste buds from different regions of the tongue. Thus, T1R1 is most prominent at the front of the tongue in the fungiform taste buds and in the palate, whereas T1R2 receptors are more prominently expressed towards the back of the tongue in the foliate and circumvallate papillae. With the T1R3 receptor, however, there seems to be a much more general distribution of this receptor in all the different types of taste bud from different regions of the tongue and palate, and this pattern of expression suggested to us that T1R3 might be co-expressed with T1R1 and T1R2 receptors. When we tested this possibility by double-labeling in situ hybridization techniques, we discovered that this was indeed the case, so we could define three classes of taste receptor cells: cells that express T1R1+3, cells that express T1R2+3, and cells that express T1R3 alone.

Given the now well defined and substantial sequence differences between human and mice T1Rs, it may not be surprising that mice do not taste a number of well-known artificial sweeteners which we readily recognize. Thus the specificity of the T1R2 sequence determines the ability to discriminate specific sweet tastes.

I cannot say anything about human mutations, but in the case of mice there are genes that map to loci involved in bitter taste. Other genes, e.g. T1RS, map to a locus involving the control of sweet taste sensitivity. We have taken the T1RS genes from animals responding to sweet tastes and found that they have specific sequence differences from the version of the gene found in non-responding (i.e. "non-taster") animals. If the responding gene is transferred into non-taster mice, then the transgenic mice are transformed into animals which respond to sweetness. So it is clear that the genetic differences between receptors do impact on behavior.

Philip James: In these transfection experiments, you imply that there is no signaling in the non-taster T1RS genetically endowed animals. If so, what happens to the neuronal system that relates to the responding animals when transfection occurs and the animals are no longer responding to the taste?

Linda Buck: We are not talking about response systems that do or do not exist. The non-taster animals have receptors that do respond to sweet compounds, but much less so than in the taster animals. Therefore, the cells are not silent.

Nick Ryba: Yes, but in some of our knockout experiments there is real silence, with the cells failing to respond at all to the signal, but the cells themselves are perfectly normal and healthy. The taste receptor cells do not have dedicated axons—rather, they are modified epithelial cells innovated by axons from the sensory ganglia. So in the taste system we are dealing with a rather different neuronal projection system from that seen in the olfactory tract.

Albertino Bigiani: We must remember that there are probably lateral interactions between taste cells, because it has been shown that in the mouse taste bud there are electrical synapses between cells, so these probably influence the response of the taste bud.

Cutberto Garza: If we consider why taste and smell are so important, it is perhaps because it allows us to cope with future events. So whether we smell something as good or bad has a consequence in behavioral terms. Has anybody looked at knockout animals to see to

what extent this alters their future behavioral responses to different tastes or odorants, or is this so far-fetched a concept because in practice we are dealing with a digital system that is integrated and it is the central processing that is critical?

Dan Storm: If we are talking about sensory inputs, then we have to realize that, from knockout mice experiments, we discover that receptors and signaling systems found in the olfactory epithelium are in practice expressed elsewhere, so there may be autonomic inputs relating to identical receptor types, about the function of which we know nothing.

David Hill: We need to be careful when thinking about taste because we are dealing with multiple pathways where a cell is not expressing only one of these pathways, there is an interplay between multiple signaling from different cells.

Nick Ryba: It is true that cells that respond to bitter stimuli respond to multiple bitter stimuli and do not mediate any responses to sweet or umami tastants?

David Hill: There are a number of studies, e.g. by Gilbertson and Smith, relating to different tastes where they used whole-cell patch clamp analyses in taste buds. They isolated the apical domain from the basal lateral membrane of a cell and then found a large percentage of their cells responding with multiple currents from a single cell in response to different stimuli.

Nick Ryba: They reported that half of their cells responded to sweet and half to bitter tastants, but 25% of these cells responded to both. This suggests that there is considerable overlap and some dual functioning. This then implies that with a specific knockout of one class of receptors, you may get unpredictable sweet and bitter responses. However, this is not what we have found—we found very specific effects with a particular knockout inducing a selective and an absolute effect.

David Hill: I am sorry, I tend to talk about salt signaling rather than sweet or bitter responses. Sodium gradients involve a very different receptor from those which Nick Ryba is talking about.

Bernd Bufe: We have to be careful before assuming that what we, humans, discern as bitter compounds are also bitter for mice and that the mice have pure tastes. At high concentrations substances may taste bitter-salty or bitter-sour to us, and the same is true of salt. Very old data suggested that salt exposure at very low concentrations induces a slightly sweet taste with saltiness coming at higher concentrations; potassium chloride, however, provides a bitter-salty taste. I would also like to add that the bitter receptors in humans are quite different from those in mice. We also have evidence of two receptor types in humans with polymorphisms which strongly influence taste sensitivities, and we do not know whether this influences the nutritional habits of humans. I accept that from the molecular data, at least at a receptor cell level, there is an organization of sweet and bitter receptor cells, but I am not convinced that patch clamp or other in vitro tissue studies necessarily relate to the taste qualities that we activate in vivo. At high millimolar concentrations of a tastant, many cells may be activated and not simply those discerned on a molecular, biological basis.

David Lin: Gilbertson and Smith have tied their detailed stimulus studies to behavioral and psycho-physiological responses in the animals, and great care has been taken to ensure that confusion is not arising because of concentration effects or a poor relationship between in vitro and behavioral responses. David Smith refers to what he terms as a "taste space" and they are not dealing with high concentrations which alter perceptions.

Bernd Bufe: Agreed, but in many patch clamp experiments high concentrations are used and I would be much more convinced if these other investigators used concentrations close to the threshold levels. To discern thresholds in rats one needs to identify an aversive behavior which by definition means that you are above the threshold level. Under such circumstances when a substance tastes predominantly sweet, but also has a bitter connotation, this implies the activation of more than one receptor cell. In humans it is much easier because you can ask them to identify thresholds, but we still in humans then have the problem of relating it to the cellular and molecular level.

Albertino Bigiani: Is Nick Ryba sure that with genetic manipulation you can clearly separate the umami taste from the salty taste? I thought that there was a paper which showed that taste cells could bind both glutamate and sodium.

Nick Ryba: The glutamate is a problem because of a pH effect. We generally use sodium glutamate and counteract the effect of sodium with ameliorite. I should emphasize that our data tell nothing about the salty taste and whether it uses the same cells or not. All we know is that the salty taste does not require the trypan 5 ion channel.

John Blundell: I have worked on the umami sense in humans and the psychological responses to it. Some colleagues deny that there is a separate umami sense. I have used glutamates and other stimuli and get a response to this particular taste. What is the consensus in the field? Is this definitely a sixth sense?

Ed Rolls: There are lots of pieces of evidence. In the cortex there are completely different sets of neurons which respond to sodium and to monosodium glutamate.

Nick Ryba: We have identified a receptor combination T1R1+T1R3 which has the characteristics relating to the umami taste, i.e. they do respond to glutamate with amplification by IMP, but also respond to a ligand that has been characterized as eliciting umami taste

LAP4. Yet the matabotropic glutamate receptor has never been shown to have responses that change in the presence of IMP and it is an abnormal type of receptor because it is missing the first half of its extracellular domain. From the crystal structure of the metabotropic glutamate receptor, we know that the first half has some absolutely essential components for the ligand binding site, e.g. the signal peptide which normally allows this extracellular domain to enter the extracellular environment. Secondly, it has about half of the ligand binding site in two domains. So the idea that this modified metabotropic glutamate receptor would be able to fold to form a functional receptor would be extremely strange. Finally, an important finding is that in knockouts of M2R4, the metabotropic glutamate receptor does not affect the ability of mice to taste glutamate.

John Blundell: I have worked with human responses to sweetness and to aspartame in particular and one of the problems is that in mice and rats aspartame is not a sensory agonist. Given the non-response of mice to this particular sweet taste, do we have an array of species-specific receptor types across the animal kingdom?

Nick Ryba: Every strain of mice that we have looked at fails to respond to aspartame and then we find at an electrophysiological receptor or behavioral level they do not respond either to a further range of artificial sweeteners. If, however, we change one of the T1R2 or T1R3 receptor cells and insert a T1R2 human version, then they will now respond to both aspartame and several other relevant tastants. The difference between the human and mouse T1R2 relates to 30% of the genome and differences in amino acid sequence in this portion make all the difference.

John Blundell: So that is just a biological accident?

Nick Ryba: We have to recognize that aspartame was something that produced sweetness for humans but there are probably other compounds which will produce a response to sweetness in mice but not in humans.

Albertino Bigiani: Where are these umami receptors located? Are they in the fungiform papillae or found throughout the tongue?

Nick Ryba: They are expressed in all taste buds but found predominantly in the fungiform papillae of mice.

Albertino Bigiani: Yet mice nerve recordings reveal that the information from umami receptors is transmitted by the glossopharyngeal nerve which innervates mainly the vallate papillae.

Nick Ryba: Mice respond much more broadly than humans to a variety of different amino acids. These are potentiated by IMP and we know these transmit primarily through the corda tympani nerves, not the glossopharyngeal nerve. We have to recognize that there may not be a good relationship between nerve recording and the behavioral response of the animal. If we look at particular knockout mice where we have restored the bitter function in the T2R cells, then with an amino acid such as isoleucine there is activation of the T1R1 and T1R3 receptors in the presence of IMP.

James Stubbs: I would like to consider PTC [phenylthiocarbamide] taster status in humans. Some investigators suggest that this status in humans is very important and influences diet selection. Yet we have heard that rodent models seem to have a predominance of bitter rather than sweet taste receptors and I wonder what the role of a PTC taster system is in humans?

Nick Ryba: Another group at NIH has suggested that a single T2R38 receptor has the PTC function and that it is a single one of these genes which explains the differences in the ability of different people to taste PTC. I think, however, that the linking of PTC taster status to dietary differences may well be an over-interpretation of the correlations observed in man.

Bernd Bufe: In this receptor there are three amino acid positions which determine whether the animal functionally senses the tastant or not.

Fat-Related Taste Responses

James Stubbs: We heard recently about possible essential fatty acid responding receptors—has this proposal been confirmed and, if so, what do we think is their nutritional role?

Ed Rolls: We know that there are normally not many free fatty acids as such in the diet, so salivary lipase would then be an important contributor to any sensing of essential fatty acids. But in humans there isn't much salivary lipase and in monkeys we have found that at a cortical level the fat-responsive processes relate to texture and not to taste. Furthermore, we find that linoleic acid is not a particularly good taste stimulant and humans don't find linoleic acid a particularly strong tastant. I think that the mechanism relates to a texture sensing system, which in turn relates to fat in the diet.

Philip James: In the texture/viscosity discussion are we dealing with something that is necessarily specifically fat-related?

Ed Rolls: I think we have to be careful because the texture channel in the mouth is separate from that relating to viscosity. We need to study the rheological properties of foods in greater detail—measures of this property are not easy: we have tried the coefficient of sliding friction, but we need to look at it in greater detail.

John Baxter: The texture of food relates to a very complex system and we need more sophisticated measurements than that relating to viscosity so that we can explain the particular liking for foods such as chocolate, mayonnaise, and cream cheese.

Philip James: So these concepts are completely

different from those relating to the fatty acid or triglyceride specific responses?

John Baxter: They are independent of the fat concentration in the food because one can manipulate the food in other ways to improve the liking for the food. Modified starches and different structural properties of foods may contribute but they do not necessarily have a great impact on the proper rheological characteristics of food which drive the liking for a product.

Albertino Bigiani: On this basis why do we not use paraffin oil instead of olive oil to season salad?

John Baxter: The chocolate industry has historically used the sheer measurements of 60 reciprocal seconds to describe the rheological characteristics of chocolate but we have discovered that testing the rheological properties at very varied sheer rates allows us to identify what determines the liking for a chocolate. Thus one can drive the liking for chocolate on the basis not of simple viscosity measurements but by other means which alter the mouth feel and the process of swallowing and chewing. These properties are of greater significance than that observed with a simple rheological measurement.

Solomon Katz: Anthropologists recognize that humans target fat and this is seen under many different circumstances. The second point is that in evolutionary terms humans in the neolithic period migrated essentially along the coastal regions eating huge amounts of seafood loaded with omega-3 essential fatty acids. It could therefore be argued that there would be no need for omega-3 fatty acid receptors because there was an abundance of these in the diets of migrating societies, and we know the importance of these EFAs for brain development. A further point relates to the taste sensitivity of humans to thiocyanate—it is recognized that this goitrogen blocks appropriate thyroid action and it can be detected very readily by individuals who have a low thyroid activity. Obviously those who lack the ability to discern the thiocyanates in the diet are much more likely to develop goiter.

James Stubbs: Le Magnen many years ago added the petroleum jelly Vaseline to the chow of rats and discovered that they developed a marked preference for this diet but that when Vaseline was removed then they did not preferentially search for Vaseline-enriched diets. Ordinary fat, however, induced a preference which persisted when the fat was removed. More recent studies have now shown that one can manipulate the sensory properties of food by mechanisms other than simply adding sugars or fats. An attractive tastant will induce an increase in intake for about 5 to 7 days, but to maintain an elevated intake one needs to have a genuine nutritional manipulation with extra sugars and fats.

Ed Rolls: Surely one could take a pure polysaccharide and alter its configuration to mimic the slick quality of fat? If this could be done, then we could begin to work out the actual sensing system.

John Baxter: There is some sensory pleasure relating to the melting property of fat itself in the mouth at body temperature because the change to a liquid form enhances the coding properties in the mouth and this is independent of the rheological properties. This melting phenomenon would be very difficult to mimic with polysaccharides. Furthermore, we have to remember that as the fat melts, there will be a release of other aromatic compounds as well.

Olfaction and the Processing of Multiple Inputs

Kjell Döving: I have been particularly interested in alarm behavior in carp. When the fish is exposed to an alarming substance, there is a typical response with the fish hiding in the mud to avoid predators. Three olfactory tracts are involved on each side of the fish and connect to different regions of the brain. In the epithelium you find the lamella and on the lamella you find the receptors which are either ciliated or microvillous receptors. The third set of receptors are in the crypt cells. The intermediate receptors in the cell bodies in the middle of the epithelium have microvilli which project to the lateral part of the brain and mediate food behavior. The crypt cells, projecting to the middle of the olfactory bulb, may be related to sexual behavior, so different morphological types of sensory neurons mediate different types of behavior.

Philip James: Are we agreed that the response by the olfactory receptor can be modulated in different ways at the post-receptor level and that this can occur before one moves up into a different level of informational processing in the brain?

Barbara Talamo: It is a bit early to say, but olfactory epithelial cells which are made to express odorant receptors that couple to endogenous signaling molecules show that there is a lot of cross-talk between the different post-receptor pathways. I am, however, unclear about the mechanism of interaction between partial agonists and antagonists in this processing. One odorant can block the activation of a receptor by another. Then one does not know how the different receptors in the axon terminal are involved in the signaling pathway or how the receptors in axons in the periphery interact in the signaling pathway.

Linda Buck: it has been very difficult to get specific antibodies to recognize these individual receptors. Over many years investigators have thought that they had suitable preparations but nothing has really been published which allows one to be confident that there are odorant receptors on axon terminals.

I just wonder if there are rapid blood flow changes

which might alter the exposure of the receptors? In rodents there is a projection from the main olfactory bulb to the amygdala so the signals, if generated in the main olfactory bulb, could go first to the amygdala and then be relayed on to the hypothalamus. I do not know if it is the same in primates, including man. One can observe rapid effects on endothelial sensitivity because very rapid effects are observed, even with intravenous estrogens.

David Lin: We may be seeing the development of new techniques which will help our imaging of cellular responses. For example, the GFP fluorescent molecule has been split with the N terminal transferred to the C terminal with an inversion of the molecule and the addition of a calmodulin-binding domain to one terminus. As calcium influxes into the cell, the calmodulin induces a conformational change in the GFP so that the cell glows green. Thus when put into muscle cells of mice, muscle contraction makes the whole muscle glow green. We are therefore hoping to use this technique in neurons and visualize them in that way.

Hanna Mustaparta: Very similar work with dyes has been undertaken on the olfactory system of insects. We find that each receptor type projects to a single unit and there are four units in the pheromone system. In practice, we discover that the optimal recording with the use of dyes is as sensitive and specific as the electrophysiological data.

Temporal Aspects of the Response to Odorants and Tastants

Barbara Talamo: I would like to suggest that receptor signaling is rather more complex and temporal aspects are important. It was shown many years ago that one can record from a signal neuron in a frog and find responses to a variety of odorants. So we have already heard that there is a combining mechanism in the sense that individual neurons expressing single receptors can respond to a number of odorants. However, the response differs depending on the odorant so there is a short burst response to one odorant but another odorant induces a burst of activity which extends beyond the end of the odorant presentation. There are even some which only start signaling at the end of the stimulus, so I wonder whether this complexity might help in the processing of information in the olfactory bulb? I wonder whether the different odorants operating at a single receptor induce different responses by activating different signaling systems, e.g. the cyclic AMP pathway or the phospholipase C pathway, which may not only influence the cyclic AMP-activated ionic channel, but influence also the adaptation to a signal, thereby activating two different pathways differentially. In pharmacological terms we could envisage a receptor that is bound in a less active state and if this applies to the olfactory responses, then there are different ways by which the odorant can influence receptor responsiveness.

Dan Storm: I would just like to comment on the complexity of the signaling system. Do not forget that when one is activating the cyclic AMP processing with odorants in the olfactory sensory bulb, we know that there is a cyclic oscillation in cyclic AMP.

Linda Buck: Barbara's emphasis on the temporal quality of responses to different odorants is important, but we do not know with this single receptor response how much of the complexity of response is important in determining the information that the brain acquires. The olfactory bulb is certainly not simply replicating and relaying the information to the brain, but is actually modifying the signaling process. There is an argument as to how much the signals are changed, modified, or refined in the olfactory bulb, but in the brain we have not even started to consider how that next processing layer is organized.

Kjell Döving: If you give the same stimulus repeatedly to a receptor cell, it will not retain the same temporal code. Secondly, in my fish experiments, there are at least 1000 receptor neurons that input to one mitral cell, so it would be very difficult to understand how a temporal code could become important for this single cell. Furthermore, in fish anyway, stimulating the mitral cells induces the same behavior with different frequencies of signaling from the olfactory tract. One sees this because the fish have a limited repertoire of behavioral responses. One specific response involves stopping swimming, going to the bottom and then swimming backwards. This behavior can be invoked with different frequencies of stimulation.

Barbara Talamo: But I thought I had understood that these were very specific receptor responses and that a very limited number of glomerulae in the fish are involved in this behavior and that different odorants do not give the same response?

Kjell Döving: I agree that this system is more specialized but it is not just a single odorant that evokes this behavior.

John Kauer: Perhaps the temporal aspect of the response governs the quality of the perception. We have to remember that most animals, including humans, have an olfactory perception that lasts no longer than a second. In rats, recorded neurons show that rats can discriminate a smell within 200 to 400 milliseconds, so this gives a feel for the temporal requirement. Then, when it comes to sensitivity issues there are good data in insects that a small number of molecules can stimulate olfaction with thresholds probably in the picomolar range, e.g. 10^{-12} or 10^{-13}. There is a variety of findings with some reports showing thresholds down to the 10^{-18} and up to 10^{-9} for exactly the same compound in the same animal. Never-

theless, I think we should be considering the picomolar range as relevant so this then demands some amplification mechanism from the primary processing of the signal through the second messenger system and up through all the brain circuits up to the cortical representation of the perception.

Christer Löfstedt: If an insect experiences two different odors and they reach the receptors at the same time, one compound may elicit an immediate spike, whereas another compound may show a slightly delayed response. This proposition is difficult to test and we should only be attempting to set up experiments where we have a realistic chance of discriminating such issues.

Hanna Mustaparta: In insects we do not see signal inhibition when stimulating receptor neurons but only a fast excitation response. Of course, there are small differences in sensitivity and temporal pattern even within the same type of receptor neurons responding to the same chemical. So we have not found in insects what is found in rats and I think we need to be careful about the generality of our experiments.

Ed Rolls: The odor system may well respond rapidly but one can still maintain the same perception to a smell: in humans a rose is a rose is a rose even after 5 seconds!

John Kauer: Agreed, but we must remember that many investigators use compounds most of which are complex mixtures and we haven't even begun to consider what is involved in a mixture involving 300 components!

Barbara Talamo: Agreed, but we have to remember that when we walk into a room full of roses we adapt rapidly so that there is no longer a recognition of the rose odor after a short while—this may be a higher order function.

Solomon Katz: The problem is even more complex if you consider various aromatics where there may be 1300 organoleptics. How we integrate that information has not yet been considered.

John Kauer: This issue of timing is intriguing because if you do experiments with rodents which are exploring an experimental open field and one provides a food source at one location, then the mouse or rat identifies the source of the food by chemotaxis far more rapidly than we could, but it involves a process lasting many seconds and up to some minutes. So the animal has to sample a gradient as a function of time which implies a very rapid and repeated turn-off of the signal to allow the negative or positive gradient to be discerned.

Linda Buck: This may occur at a higher level but some of the adaptation appears to involve cellular pathways in the olfactory sensory neuron, e.g., the cyclic nucleotide gated channels. For some years there has been a series of papers proposing a temporal coding in the responses of olfactory sensory neurons. Experiments on honey bees, for example, showed they were able to discriminate odorants unless they were almost identical, and temporal coding was not required for this discrimination except among highly related compounds and this discrimination may involve lateral inhibition by contiguous neurons.

John Kauer: What we have seen is a change in firing pattern over the time course of a single stimulus and there are multiple different firing patterns in many different cells occurring simultaneously with the onset of a particular odor coming into the nose. That is one kind of temporal coding. Another aspect is the degree to which the firing of these neurons are linked and perhaps synchronously activated so that one neuron is responding having taken account of collateral activity. I am attracted by the idea that mathematically there may be, let us say, 1000 different receptors expressed and if they are either turned on or off with a particular odor, then you already have an unbelievable number of possible configurations coming from the 1000 receptors without having to infer any temporal firing significance. The degrees of freedom are therefore phenomenal.

Christer Löfstedt: The issue of higher cerebral processing is also involved because if a male rat is exposed to the urine of females without having been in the company of females before, then they are unresponsive. But once they have been with a female for 24 hours, then the presence of female urine induces remarkable behavioral changes, which implies that the interpretation of the signal has totally changed.

Technical Issues When Monitoring In Vivo the Central Responses

Philip James: Let us now deal with the issue of the validity of our new analyses which specify particular regions of the brain as it monitors and responds to these sensory inputs. Ed Rolls, please reassure us that you have sufficient specificity and reproducibility in your new scanning techniques for monitoring neuronal activity in the brain as it responds to olfactory and taste inputs.

Ed Rolls: When one is considering a single study it is important to undertake studies on about 12 subjects so that one can do group analyses and assess the consistency of response across the individuals. One can also perform random effects analyses, which reveal whether a particular response in the brain is random or not. The issue of consistency is, however, important and I have just completed a meta-analysis of all the imaging studies ever undertaken on taste and olfaction and collated the 278 foci which have been proposed as responsive areas. The majority of studies on the orbito-frontal cortex identify the medial orbito-frontal area as being important for responding to pleasant representations of these olfac-

tory and taste inputs, whereas the lateral orbito-frontal area responds to the unpleasant inputs. The insular area does crop up very regularly as the cortex relating to primary taste in most people's studies. Yet we are still in the process of discovering, for example, the recent finding that this insular area is not activated by olfaction. So I agree that we have to be careful, compare lots of studies, each with substantial numbers of individuals and then undertake rigorous analyses for random effects.

The second point you are raising is the issue of what we are measuring. You have to understand that we are measuring something that is relatively crude and with a low resolution. Thus in focal magnetic resonance imaging, we are monitoring the response in blood oxygenation levels—when part of the brain is activated with synaptic activity there are substantial ionic shifts across the membranes which sets up a metabolic demand which in turn activates the vasodilatory response with more red cells and oxygenated hemoglobin going through this area. So an activated area is measuring the change in deoxy-hemoglobin going through the activated area because it is paramagnetic, and we are measuring something that affects the metabolism of the local brain area. Your third point on the resolution of the MRIs is important because we are rarely more precise than to within 3 mm.

My final point relates to how we integrate these MRI analyses. Our work on single neurons and their responsiveness specifies what is happening in a very specific way but the MRI studies are then necessary to identify areas involving numerous neurons which then relate in a monkey to its behavioral response. If we can see parallels between humans and monkeys in the identification of selective areas and their responses then we can use the single cell technique to link specific information on neurons to human behavior.

Solomon Katz: There are further dimensions to these different approaches—thus one can look at particular receptor responses, e.g. serotoninergic responses, to relate to pleasure. Neuroepinepherine receptor analyses have already been undertaken.

Linda Buck: How many neurons have to be activated before you see a bold signal on MRI? For example, can you see ocular dominance columns and can you see changes in directional sensitivity in the monkey cortex?

Ed Rolls: Yes, one can use a higher MRI field with local head coils so that you can get the resolution down to about 0.5 mm, so it has been possible to see ocular columns which are about 0.5 of a millimeter apart, but I am not sure that anyone has observed directional sensitivity. In terms of the proportions of neurons affected, then if there are parallels in response to visual stimuli where there is no associated pleasant or unpleasant responses as seen with taste, then we know that only about 3% of the monkey's orbito-frontal cortex neurons respond to a discrete stimulus. Now in humans we can pick up a signal that relates specifically to the reversal event after the end of the stimulus, but we do not have direct comparisons between monkey and man. In essence we are talking about some tens of thousands of neurons operating per cubic millimeter of cortex. This means that with MRI at its present resolution, you cannot hope to discriminate a spatial representation of some image or variety of taste responses. We know that each neuron carries independent information to a small number of other neurons, so the neurons are the computing elements of the brain, and in understanding any observed spatial representation you will need to know precisely what individual neurons are responding to. Thus the MRI is never going to help us with that. The beautiful MRI pictures are not a substitute for studies of single or multiple neuronal physiology.

Barry Keverne: You can increase the sensitivity of FMRI by increasing the power of the magnet. In Cal Tech they have a 12-tesslar magnet where they can actually monitor individual cells, but you cannot put humans in such a powerful magnet.

Ivanka Savic-Berglund: We have found problems in assessing with higher-powered magnets the region dealing with olfaction because there is a substantial loss of signal because of the variety of tissues near this cortical area, so I doubt if increasing the power of the magnet will help much in humans.

Ed Rolls: There are problems arising because of the air-filled sinuses, but there are ways to get around the difficulty, e.g. imaging in the vertical plane so that one can minimize the effect of these sinuses. Another trick involves putting a piece of dio-magnetic material into the mouth which helps to linearize the field, but it is not very comfortable!

Ivanka Savic-Berglund: When we measure receptor responses by PET scanning, we find a variability of about 10% in the apparent density of receptor responses. Now if one undertakes blood-flow studies in the brain monitoring various activation processes, then one has to recognize that activation in an area induces a change in cerebral blood flow of about 4% to 5%, so the signal-to-noise ratio is very low and therefore multiple repetitive studies are required. PET scanning, of course, cannot be used in this repetitive mode because of the problems of radioactive exposure. Where a few repeat studies have been undertaken, there is a high reproducibility. However, this reproducibility depends upon the filtering that you assign to the signals, so with a 10-mm signal, you have higher reproducibility because you are scanning a larger area. PET should not be used if one is looking for a high temporal resolution. Thus with olfactory studies one initially activates one area, but several other regions

will subsequently respond; therefore, PET, with a time resolution of 60 seconds, will not pick up this sequence. On the other hand, PET is very good at monitoring limbic systems or other areas of the brain where FMRI scanning presents difficulty because of the juxtaposition of air, bone, and brain tissue.

Barry Keverne: How reliable is PET scanning when one is assessing the cerebral response to a reward? If one has to scan individuals a number of times, then we know that the level of that reward response can vary during the time of the repeated scanning. One of the areas which does not seem to come through on the FMRI scans but does show up in rodents or monkeys in Wolfram Schultz's work, is that the ventral striatum seems to be very important; the dopamine pathways seem to be very important in responding to reward but this does not involve the ventral striatum.

Ed Rolls: One of the good things about FMRI is that you can complete the stimulus a number of times, thereby obtaining statistical power, but in addition, if one is considering the intensity or pleasantness of a stimulus, then one can undertake studies with humans where one can correlate the degree of brain activation with the variation in reported pleasantness. This is then a powerful design and one can correlate the brain activation and the subjective response. Unfortunately, with PET scanning, one cannot really undertake repeated measures and that is why we have concentrated on MRI where one can literally undertake studies many, many times in individuals as well as the same group of subjects.

Cerebral Processing of Taste and Smell

Cutberto Garza: In considering the specificity of information in the brain, is it reasonable to consider olfaction and taste as probably the two best systems for beginning to understand the parallel processing and, indeed, integrative activity of the brain? Are there promising techniques being developed where we can go beyond the monitoring of single neurons and overcome the disadvantages of PET scanning? Are there techniques emerging which provide higher spatial and temporal resolution?

Ed Rolls: The system of taste is certainly valuable in helping us to understand the way in which the brain computes because the system for taste is incredibly simple in terms of its cortical representation. In effect, the brain acts to categorize taste and these categories operate in a simple, competitive network which is one of the basic operating systems relating to the cerebral function. Smell is a bit more complicated because there is evidence that in the olfactory processing the time of signaling is potentially important. Thus, by the time the signal reaches the secondary olfactory cortex, there may be a time-dependent difference in the encoding of the neurons firing in response to this signal thereby producing an almost exponential decline in firing as another stimulus comes in. So if one studies a single neuron, it will have a very high response to one stimulus, but a not so high response to a second, with a progressively lower response to all subsequent stimuli. This certainly happens in vision so you are setting up a coding system where a neuron will continue to respond to different stimuli and distribute signals appropriately, but a single activation process of neurons can be defined by the exponential decline in its firing rate to a single type of signal. So olfaction is still relatively simple but with the numerous neurons firing in response to different sensory phenomena, one might have up to a 1000 independent, dimensional responses. Then one probably has a coactivation of neurons, which provides a conjoint representation of the overall input, and this is what we then discriminate. This is a device for competitive learning about the nature of the olfactory input. The visual processing may be more complicated because a stimulus hits one bit of the retina, but somehow you have to recognize that it is the same object when that visual stimulus sweeps across other parts of the retina as the object moves or is shown in a different orientation or view. Taste is therefore very simple, but the multiple inputs of olfaction make it more like vision where one has to integrate and have the ability to identify in effect the same input from the outside world, operating through several channels. This is called invariance.

John Kauer: I don't think we should fall into the trap of thinking of imaging in terms of phrenology, i.e. where, in keeping with our longstanding view of the brain, we consider it as an anatomical entity where different elements can be linked with specific functions. Secondly, the cortical distribution of olfactory inputs is going to turn out to be a very common form of information processing and we are going to find that other brain areas are involved in a level of analysis which we as yet do not understand. In terms of new techniques, I think that the new emphasis on using multiple electrodes with simultaneous recording is going to prove helpful. Having multiple electrodes monitoring specific neurons in normal responding animals is, although useful, extremely technical. Now we are attempting to use dyes, discernible by imaging techniques which respond to voltage fluctuations and therefore to ionic flux and thereby providing direct measures of neuronal activity with millisecond resolution. The dyes are taken up by the neuronal membranes and are fluorescent so that they change their fluorescence depending on the change in charge across the membrane. The problem is that you cannot look much deeper than a couple of millimeters of cortex, but there are now classes of dyes which are being developed which are excited within the infrared wavelengths. These

may well allow us to undertake a tomographic reconstruction in vivo in different parts of the brain. This still means at present that the brain has to be surgically exposed in the specific area of the visual cortex or olfactory bulb or other area of interest, but the animal can still be completely responsive despite this intervention. Finally, I think I would consider that the olfactory processing is rather more complicated than you imply because an animal or human can identify the odor signature of a particular compound in a variety of different backgrounds of olfactory stimulus. This discrimination, I think, is probably handled differently from the exquisite but clear system evident in visual recognition processes.

Ed Rolls: Another issue relates to the temporal nature of stimuli and whether or not cells are synchronized or have different temporal profiles. One could consider information theory and then separate out the neuronal activity from a number of spikes, the time profile of the firing and whether the co-firing of different neurons might indicate a specific stimulus. My hunch is that this issue of temporal coding is difficult for the brain to hold in its informational system without putting it into some coding on the rate in the primary or secondary cortex. This is certainly consistent with what we see for vision.

Ivanka Savic-Berglund: I deal with patents with epilepsy, some of whom who have a very stereotyped, repetitive seizure involving the induction of the same type of very unpleasant smell which is never defined. This seizure starts in a tiny area involving perhaps more than 30,000 neurons. The same unpleasant smell is consistently evident with the seizure, which begins in the pre-frontal cortex within the inner part of the temporal lobe around the amygdula.

Linda Buck: I guess the seizure involves many neurons and therefore many different kinds of smell, so perhaps that is why it is so unpleasant?

John Kauer: I have also heard that people who have uncal seizures invariably report horrible smells often simulating that of burning rubber.

Cutberto Garza: If one takes the visual system as an example, then we know that the visual system provides a lot of information only some of which we use to obtain a picture that is considered significant. If the same is true with olfactory and taste, how will we ever distinguish what is important because there is discrimination and some, if not many, of the signals may be ignored centrally?

Philip James: In this week's *Nature* journal (Pelli DG, Farell B, Moore DC. The remarkable inefficiency of word recognition. Nature. 2003;423:752-756), this feature is beautifully illustrated with a visual series of dots which one has to consider over perhaps a minute before one suddenly realizes that there is a picture within the dots. So the implications are that there is a lot of sifting in the visual dimension and an enormous amount of integrative activity before we identify a significant image.

Linda Buck: Agreed—and in relation to olfaction, the question is how do we see a complete change in a perceived odor when there is a small change in the type of receptors responding to it? Is it possible that some receptor types convey a particular odor strongly whereas others do not? Perhaps we are involving minor discrimination to make these judgements. Or is it possible that one or two receptor types only would be able to convey, for example, the rancid, sweaty odor?

Ed Rolls: we are discussing essentially the bottom-up versus the top-down options for discrimination. In visual terms one can present an image for 20 milliseconds and then stop the neurons firing by what is termed a "backward mask" so that there is not time for the information to go all the way up to some higher area and for it then to return to begin to account for the firing of neurons. We have been able to show that those neurons which selectively discriminate facial features can fire within 20 milliseconds and the image is made so there is no back projection and modulation of incoming stimuli. Obviously one can have top-down effects in olfaction—we all know that the color of wine influences what wine tasters tell us about the quality of odor and taste, so there is plenty of opportunity for top-down processing.

A different issue relates to capacity and whether we can respond to a huge number of odors. We tend to assume that there is no noise in the system but as soon as there is some noise in the informational processing, then one has a problem. So the amount of information each neuron may provide, e.g., 0.05 information bits, requires that many receptors should be involved and a single receptor is not necessarily giving a perfect signal each time with unique significance for an odor. In this case we may be talking about a linear scaling of neuronal inputs but in informational processing we need to consider log measures with an exponential encoding to cope with different stimuli or odors. When these calculations have been made, the cortical capacity is in practice sufficient to cope with a remarkable number of inputs so the key to the processing may not be cortical capacity but the number of receptors involved.

Behavioral Programming

Gunnar Bergström: I would like to ask a general question relating to the inheritance of particular responses to odors and tastants. I understand that an absolutely newborn babe automatically attempts to seek the breast of the mother and that an unwashed breast is preferred, thereby implying that some odor is involved. I am also told that vanilla is a component of the smell of human breast milk—do we know what the evidence is?

A further example of what appear to be intrinsic responses relate to smoke and the fact that for millennia animals and man have been exposed to the risks of fire and need to take early avoiding action. Is there any evidence of an intrinsic alert process stimulated by smoke?

Lars Hanson: The newborn has a number of inbuilt mechanisms for actively searching for the breast but we do not know whether this recognition system is acquired in utero or is an intrinsic phenomenon.

Philip James: I thought there were experiments whereby the feeding of the mother with particular foods, e.g. banana, would then condition the newborn baby to accept immediately a banana-flavored food.

Ed Rolls: Yes, it is a well-established phenomenon.

Kjell Döving: Studies on breastfeeding in rabbits show that the young feed for an extremely short period—one to two minutes a day—and that the babies search for the breast. A group in Dijon recently identified the odorant as methylbutanemaldehyde.

John Kauer: Some very nice experiments on in utero conditioning by Blass and Petersen show that a rat pup would not attach to the nipple if the nipple was washed. Then I understand an odorant such as citrol, on being injected into the amniotic fluid, would then induce the newborn pup only to attach to the breast if it was painted with citrol. This is a very explicit example of in utero training.

Olle Hernell: Is anything known about the importance of introducing particular foods and tastes when weaning? In other words, is there a window in the timing needed which may determine the tolerance or intolerance to a new food? This could be important if there were real benefit in introducing particular tastes to babies aged 4 to 6 months.

Irv Rosenberg: It seems to me that we are trying to understand how particular tastes or smells lead to behaviors which are useful or protective in evolutionary terms. When considering David Hill's views on salt taste, it seems to me that that is concerned with protecting the body fluid volume and that this is part of an important need to keep the tonicity of plasma within a tight range. An experiment has shown that a sudden drop in blood volume induced by a diuretic in female dams will induce in the pups after birth a higher taste sensitivity to salt as if they are programmed to protect their fluid volume with greater tenacity. So this may be a much more general programming of behavior very early or even in utero events than we thought.

David Hill: The concept of programming the sensitivity to salt in offspring only 10 days after conception relates to the alteration in physiological systems rather than the taste system alone. The central process may be specific for taste but the primary input relates to general physiology. So inducing sodium depletion in utero does produce higher sodium appetite in the offspring, and the more times the depletion in utero, the greater the appetite in the offspring. I suspect that if we had manipulated other systems, perhaps protein intake, or induced a change in a major hormone early in development, then we would see the same type of response. I just do not think that it is simply a specific effect of, for example, sodium deprivation per se which then leads to a selective sensory stimulus. The peripheral taste system is reversible so that at any time in development unlike the visual system you can reverse the changes by feeding a suitable, e.g. sodium-enriched, diet.

Philip James: What do you mean by peripheral? Taste buds?

David Hill: Yes, what I am saying is that the time involved is that of the full life cycle of one of the taste buds, so you do not get an immediate effect, but have to wait for 10 days. It looks as though we generate a new population of taste receptor cells which are responding to the earlier impact of sodium intake. The central nervous system is not reversible, with the physiological setting in relation to sodium fixed at that level from then on. So there is a very narrow window in dietary change very early on in life and this has permanent effects on the way the central nervous system is organized and affects water balance.

Ed Rolls: I think we have to consider two phenomena: if I am water-deprived, my blood volume drops and this induces a sodium appetite over a period of about 24 hours through the induction of the renin-angiotensin system. It would appear that this is what is involved in David Hill's analyses, and it would also appear that there is some sensitization of that system which induces an alteration in sodium appetite in utero. The second system is that relating to osmotic pressure. Thus, if I am water-deprived, not only does my blood volume drop, but my osmotic pressure goes up. This then simply increases the pleasantness of the taste of water and does not make sodium more pleasant because this would be maladaptive in terms of the osmotic effect. There are lots of neurons which are tuned to the taste of water in the mouth and these connect to the primary and secondary cortex. It is not clear how, but the thirst-induced taste for water is a secondary cortex response.

Nick Ryba: There could be receptors for water and for salt or other receptors which are responding to changes in the concentration of particular molecules in saliva with effects which are modified by the diluting effect of ingested water. So this is an indirect water-sensing system rather than a specific receptor mechanism for H_2O as such.

Ed Rolls: I agree that when you put water into the mouth, you are diluting the viscosity of saliva and if this

does not involve a taste receptor being activated, then perhaps the brain recognizes the input of water as such.

Olle Hernell: If you look at a newborn that is being exposed to human milk, then we must recognize that the sodium content of human milk is low and the newborn infant does not like too much salt, which is also, of course, dangerous. So I am wondering how relevant this early programming is to the human condition?

Cutberto Garza: Could I make a plea for some specificity in our terminology? Programming, by its very nature, is reversible, but we need to distinguish this from the biology of imprinting, which is not reversible and the mechanisms may be quite distinct. I therefore think we should specify reversible phenomena as programming, whereas we should use the term "imprinting" if it is irreversible. Many years ago, we published data on the aldosterone and renin levels of breastfed and formula-fed infants at a time when these formulae has much higher levels of sodium. We finally realized that the range of aldosterone levels in our breastfed infants were normal and that the formula-fed babies had abnormal levels, despite these levels being taken in the literature as the appropriate concentrations for the newborn! We therefore had to re-establish the definition of "normality." I have often wondered what these high sodium intakes did in terms of entraining physiological mechanisms, for example, relating to colonic function and whether this could, in part, explain the better effects of breastfeeding in diarrheal disease? The different hormonal milieu may explain their greater ability to conserve sodium. I think David Hill is therefore absolutely right to think in broad terms and not just focus on sodium, and that we are dealing with the fundamental imprinting of a variety of signals.

James Stubbs: Can these early effects, through imprinting, influence food preferences or appetite much later in life? In other words, do events in utero actually affect an adult animal's behavior?

David Hill: Julie Minnella and Gerry Beecham have done a lot of work on this showing that exposure to high-sodium diets post-natally induces a greater preference for sodium over the next 2 to 3 years.

Philip James: There is a beautiful Dutch study (Geleijnse JM, Hofman A, Witteman JCM, Hazebroek AAJM, Valkenburg HA and Grobbee DE. Long-term effects of neonatal sodium restriction on blood pressure. Hypertension. 1996;29:913-917) on formula-fed babies, one group being assigned to a lower sodium concentration for six months. Fifteen years later, those children who had received the higher sodium formulation for the first six months of life had the higher blood pressures. I now suddenly realize that this effect might relate to a selective imprinting of the responsiveness of the hormonal system of, for example, the renin-angotensinogen system, or to an early behavioral learning phenomenon, which then means that they prefer higher sodium intakes. I wonder whether the fact that Italian and Greek children seemed, at least in the past, to eat lots of different vegetables and fruits with equanimity related to the mothers having exposed their children through the amniotic fluid to these flavors and odors which are considered strange in British children. Data show that in England, young children have to be exposed in appropriate circumstances to multiple—i.e. 10 to 15—offerings of unusual vegetables and fruits before they become accustomed to the tastes and then accept them readily.

Irv Rosenberg: Are there any parallels with the relationship of sodium and the thirst for water based on blood volume changes and tonicity but now relating to sugar or sweet tastes? Do these relate to some hormonal response and what about aversive or bitter tastes—are we dealing with some imprinting or programming effects?

Christian Drevon: It is strange that we see such different preferences for foods in different cultures. If you go to Iceland, one can readily buy snacks consisting of dried, rancid fish which smell strongly as soon as one opens the packet and both children and adults love them. Yet, when presented to people from other European countries, they immediately reject them. Is this a simple learning process or something much more biological? The Inuits in Greenland eat dried blubber that is also very rancid and which is again considered as a delicacy. They can eat huge amounts of this food.

Ed Rolls: This can be simply a conditioning process because Hugh Sinclair, the famous Oxford nutritionist, lived on blubber for months on end.

Christer Löfstedt: It seems to me from personal experience that children in a modern setting become so difficult about foods that you would consider their food choices as maladaptive in evolutionary terms. I wonder whether, given the loss of 1% of our olfactory capacity per year, children are too sensitive in early years.

Solomon Katz: It is well-known that the Chinese deliberately avoid cheese because of its rancid smell and they can immediately identify a Westerner because they secrete odorants from the cheese they have consumed. Then if we think about the aversive response to bitter tastes, we have to question whether the processing from these receptors is different in survival terms. Then, when it comes to breast milk, we recognize that lactose is the sweetening sugar in the milk, but I wonder whether the sensing is different in individuals or groups with lactase or sucrase deficiency as happens commonly amongst the Inuit.

Linda Buck: I have read that the bitter taste is a naturally aversive phenomenon, geared in evolutionary terms to protect animals from poisonous plants, many of

which are bitter. I understand the same applies to newborn humans.

Irv Rosenberg: Is the aversive taste a more powerful influence on behavior and food intake than attractive tastants?

Cutberto Garza: I would like to return to the issue of whether salt responses can be generalized to other nutrients. I think salt, energy intake, and water are driven physiologically in specific ways to correct deficits, but I don't know of any other physiological systems, so people who are iron or niacin deficient do not develop specific behaviors to compensate.

Gunner Hall: As a food scientist, how do we get people to change their perceptions so that they now like previously rejected foods?

John Blundell: In answering this I would like to point out that humans are naturally omnivores so the whole world is a potentially edible restaurant! Therefore, taste and olfaction become important to guide humans to foods that are beneficial rather than detrimental. In addition to behavioral responses, it seems that these processes also generate pleasure associated with consumption. My impression is that only sweet foods are universally regarded as pleasurable, unlike, for example, the rancid foods. There are many habits in different cultures which are considered offensive and aversive elsewhere, so this highlights the importance of learning and the conditioning of humans to particular environments of foods. Thus perhaps a physiological learning underlies the connection between a food choice and the feeling of pleasure that that food has previously induced.

Philip James: Linda Buck has told us that mice have about 1000 odorant receptors, whereas humans have about 350. Then Barry Keverne has presented an evolutionary concept which implies that the processing, learning, and integrative analogies relating to food are more important in higher animals. I wonder whether omnivores have a different sensorium than, for example, ruminants? I understand that deer, dependent upon eating grass and different herbs, can smell a man at great distances if they are down-wind. So in some parts of the animal kingdom there seems to be an exquisite sensitivity to particular odorants, whereas I thought that omnivores had cruder sensitivities.

Barry Keverne: One finds that anencephalic neonates reject bitter liquids, but accept sweet tastes, so this must be a brain stem function. I agree with John Blundell that we must be careful when considering human evolution because for human survival they are highly exploratory and this is of great survival value. Thus we go through extraordinary learning processes which we then pass on through cultural means, presumably, to the next generation. I would be very surprised if the responses in adults were anything other than a learned or conditioned process.

James Stubbs: I agree that learning in humans is paramount, but omnivores have, in evolutionary terms, the system of a large-bodied, fruit-eating primate, with the appropriate teeth and intestines. The only aberrations relate to the inability to digest lactose. There is therefore no need to have receptors in terms of specific physiological stimuli because if we eat inappropriately, we rapidly learn from the gut effects what not to eat! Therefore we can extend the aversive response and suggest that this is far more immediate than potentially beneficial effects, simply because humans recognize that they cannot make the same mistake too many times. The cost of making a mistake relating to something pleasant is not that great.

David Hill: If you look at mesencephalic animals and the changes during development, then the responses to salt, sweet, and acid stimuli in rodents are there from the beginning. Indeed, the response to bitterness is even clearer than in adults. Babies are virtually insensitive to sodium to birth, but over the first couple of years, they acquire their preference for sodium. Hamsters, however, show that none of the adults prefer sodium, unlike other rodents. Indeed, they display aversion and if you do neural recordings on developing hamsters, they have a marked response to sodium early in development, but this then decreases. So it is just the mirror image of what happens in man. Perhaps if a young hamster ingests sodium, they cannot handle it, whereas a human or similar animal will respond very differently.

Gunnar Bergström: If one considers the pyrazines to which humans are so sensitive, then we have to recognize that the pyrazines which give the wonderful smell of newly baked bread are universally agreeable to man.

Philip James: I know that in testing the pork samples from pigs that have not been castrated, there is more androsteinedione and there is then a remarkable difference in the intrinsic capacity of people to smell this hormone. It is well-recognized that some individuals, particularly women, are able to pick up the smell at phenomenally low concentrations.

John Kauer: It is true that about half the male population seems unable to discern androsteindione but on continued exposure over a course of some weeks, there is an increase in sensitivity?

Gunnar Bergström: In Oliver Sachs' book, *The Man Who Mistook His Wife for a Hat*, a medical student studying intensively for exams was said to develop a remarkable capacity for smell.

Linda Buck: I think he must have taken a drug to enhance his performance and he therefore became temporarily hypersensitive. Drugs are well known to modulate taste and smell.

Dan Storm: I would like to follow up the comments on androsteindione. There seem to be a limited number of odorants that show this extraordinary sensitivity. The only other one I know of is isovalerate.

Ed Rolls: I think we have basically tastes for sweet, salt, bitter, sour, and umami tastes to which you can add the sense of water, and these are primary tastes which are innate or "unled reinforcers." This means that the first time you make that taste of importance to an animal, e.g. by sodium or food depletion, then the food-depleted animal chooses the sweet taste, and the sodium-depleted animal the salty-tasting food. This can have profound implications because it means that the brain is organized with taste pathways specified not only at the receptor level, but through synaptic processes within the brain to allow the decoding of the stimulus and the recognition of need. It is the upper brain which responds in terms of the attributes of these tastes, so I think there will be a huge burst in taste research which will reveal selective transfer systems into the brain but that this sensory loop is subject to the sort of conditioning that John Blundell and James Stubbs are discussing. Thus the conditioned appetite is a post-ingestive consequence but you also have learned aversions in response to feeling sick. Aversion is also induced through conditioned satiety, so if a substantial energy supply is associated with a particular texture or flavor on previous occasions, then we automatically become conditioned to reduce the intake of this food if we are satiated.

It seems to me that odor is very different, with very little organized within us on an innate basis, and it seems to me that we learn most, if not all, about the significance of smells. There are some obvious exceptions, for example pheromones and maybe a putrefying odor. But my guess is that food-related odors are those that we learn in association with taste and therefore associate with a pleasurable sensation. Learned preferences for, and conditioned satiety against, particular odorous foods are therefore a major learning process. The classic experiments by David Booth showed this. If provided with two sorts of sandwiches of different energy content and with different flavors, people subconsciously, after three days or so, are eating more of those sandwiches with less energy. Once this has been learned, if the energy content is covertly switched, then the individual chooses on the basis of the flavor and it take a further day or two before a further adjustment in sandwich choice is made. So we learn to predict the energy content of a sandwich from its particular flavor. This has profound implications for the food industry because they can produce very palatable foods which have an unusual energy content, but there is still a conditioning attempt by humans to consume the appropriate energy.

Linda Buck: Is it not true that aversion leads to a much more rapid response, so that an animal or human who becomes sick after a particular food immediately learns to avoid that food or smell?

John Blundell: Yes, there are good reasons why taste aversion should be so rapidly induced—it is a survival mechanism and these aversions are very enduring. Ed Rolls has described a particular paradigm linking taste or odor with the consequences of food consumption under tightly controlled conditions, but we then have to consider the application of these findings to the normal environment, where the repertoire of food is changing so rapidly. This implies that the conditioning mechanism confronted with an array of constantly changing foods is thrown into chaos so that the predictive power of the taste or odor is negligible. I think this is the problem for modern human societies where the potency of learning and the value of the predictive conditioning to guide consumption has to become less important than in our original evolutionary settings.

John Kauer: I think we have to distinguish taste from smell. Dan Storm and Kjell Döving in their experimental work have both shown a change in the set point or the sensitivity of the cellular receptors to tastants. The changes occur over a longer time frame than the training or learning phenomena that we are talking about. Then we have a further and longer time-base for the changing food preferences and eating behavior based on evolutionary stresses. The huge repertoire of olfactory receptors provide a sort of sounding board which allow us to continually change our preferences.

Cutberto Garza: Aversion is not an intrinsic phenomenon. Thus, in oncology wards, we have to be careful not to provide children with their favorite foods at the time when they are receiving a dose of chemotherapy, because they will develop a very strong, and potentially permanent, aversion to that favorite food.

James Stubbs: In the farm animal literature, they have discovered that the speed and the strength of the learning process for selecting particular grasses or foods is dependent on the magnitude of the physiological effect. Thus the color and the smell of a particular item relating to a physiological stimulus is learned, and this is very important in relation to energy intake. It would be interesting to know if the sales of aspartame-sweetened soft drinks would be much less than the sugar-containing drinks in conditions of energy deprivation, whereas aspartame-sweetened drinks may be preferred in our environment where most of us are gaining weightt?

Stephan Rössner: I run one of the few obesity clinics in Sweden and work with extremely fat patients. I am inundated with patients who describe themselves as sugar addicts. I do not know what this means except that they do focus on carbohydrate-rich foods. Some clinics focus on sugar addicts who may consume 12 liters of soft

drinks (with 10% sugar) a day. When I then ask my patients, and young men in particular, why they could not choose aspartame-sweetened soft drinks, they recognize intellectually that they should not be consuming these vast amounts, but they find it impossible to switch. This question of whether sugar addiction is a biological feature is important in economic terms because they are being referred to clinics where the care costs a great deal in terms of management.

Kaare Norum: The alteration in taste sensitivity in humans, for example, to salt, is clear. Thus, if you take a patient with hypertension who may be eating 10 g of salt a day and put them on practically a salt-free diet for 2 to 3 weeks, then they are subsequently satisfied with only 4 to 5 g of salt in their diet. So I guess this implies that the taste buds have turned over within those 2 to 3 weeks and they have adapted or been reprogrammed. Does the same happen with sugar? Can you put them on a sugar-free diet and then try to obtain the same change in preferences?

Philip James: I am told that when sugar is removed from one's regular cups of tea, then one subsequently avoids sugar-containing tea.

Ed Rolls: In terms of sweetness, I don't think we are dealing with thresholds, but with an hedonic switch.

Philip James: So if I undertake electrophysiological or NMR scanning of the sensory arcs, I should detect precisely the same responses in sugar perception until I monitor the secondary pleasurable responses in the brain?

Ed Rolls: Exactly—the primary taste cortex will be responding as usual and the rate and intensity of the sensory input from sweetness will the same whether you are hungry or satiated. The big swing will then be in the pleasantness or the hedonistic response.

James Stubbs: We haven't published this yet, but have undertaken a series of studies with different rates of energy deficit to reduce people's weight by 5% or 10%. When we do this with lean individuals, they become very aware of the sensory features of their diet and can taste lower concentrations of sweet and salt tastants. The obese, however, do not show any increased sensibility to sensory discrimination.

John Blundell: We should probably consider sweetness as a special case because it is such a potent psychobiological stimulant with an in-built preference. Almost all animals have this pleasurable response with, for example, primitive tribes going to great lengths and danger to acquire honey, even though their nutritional needs have been met. Sweetness is a commodity that gives pleasure whether or not you are hungry or satiated.

Ed Rolls: So what would happen if we could block the sensation of sweetness as a treatment for obesity? One would need something which blocked the sweet sensation for a 24-hour period.

Cutberto Garza: How much of this response to sweeteners is opioid in origin? The opioid feature does seem to be important because it can be blocked by an opioid antagonist, e.g. naloxone. We know, for example, that children when being circumcized, will not respond to pain so much if you put a drop or two of sucrose on their tongues—it is almost a homeopathic dose, but has an enormous calming influence. Can we distinguish these from the other hedonic responses to food?

Ed Rolls: This issue of opioid-mediated responses can be a myth. For example, the high you get after exercise is really not much affected by naloxone.

Barry Keverne: The point about opioids is relevant because they do reinforce pleasure and it involves a part of the brain, the nucleus circumbens and this involves two distinct transmitters—one opioid and the other dopamine. I think that there is a real possibility that we ought to be looking at the base of the striatum because so many human lifestyles seem to become addictive. Some people even become addicted to earning money when it shouldn't mean anything more to them, so I do believe we ought to be considering the possibility of an addiction to sweet substances. In my experience with all the animal literature, I find that imprinting invariably occurs at a critical period associated with a life event such as birth, mating, parturition, or sickness. They are all associated with those very specific events in life which are threatening, pleasurable, or have long-term consequences.

Ed Rolls: I agree that the nucleus circumbens is important, but where does it get its inputs from? Because that is the key issue. They come from the orbito-frontal cortex and the amygdala. In drug addiction the effects occur after the locus for computing sensory-specific satiety and that may be why drugs are so addictive because they are acting on the ventral striatum directly, thereby bypassing the orbito-frontal cortex and amygdala region.

John Blundell: We need to be careful about using the term "addiction" in relation to sugar. We can use the word addiction as a metaphor indicating that individuals find a particular form of behavior compelling. In cocaine and heroin addiction there are well-recognized brain changes and the involvement of dopamine receptors and the nucleus circumbens. So it does not surprise me that people remark that sugar is addictive because sweetness is an extremely psychobiological phenomenon producing a potent sense of pleasure. This will be coded in hedonic circuitry with dopamine, opioids, glutamates, and the cannabinoid receptors involved, and sweetness does seem to stimulate the opioid pathways more than other stimuli.

James Stubbs: Peter Rogers suggests that it is not

sugar addiction as such, but that people feel that they have become dependent on particular dietary inputs. This is not the same as the major effects of true addiction. When we undertake studies at the Rowett Research Institute with rats and provide them with the clinical liquid supplement of Ensure, which is full of sugar and chocolate flavoring with about 30% fat by energy, then they take large amounts of it and become obese. When the diet is removed, they become very irritable and show all the behavioral traits of an unhappy rat. Their body weight begins to drift down and this continues for several days before another system kicks in to increase their food intake and defend their body weight.

Aging and Its Effect on Taste and Smell

Philip James: Can we now come to whether there is a true biological aging in our olfaction?

Barbara Talamo: There is a loss of olfactory receptors from the nose with the sensory neurons being replaced by respiratory epithelium. The stem cells or basal progenitor cells for neuronal replacement seem to have been destroyed. The cycle time for the olfactory receptor cells is about 90 days, and for the taste cells about 10 days, but there are different turnover rates for different species. I am not sure who proposed the particular turnover time of this work.

Linda Buck: I have also read that the reduction in olfaction in advanced years results from a loss of the appropriate sensory epithelium, but not everybody seems to lose it. Thus, some old people have a well-preserved sense of smell.

Dan Storm: I think the data came from looking at age-matched controls for patients with Alzheimer's and Parkinson's disease. If you are 50 years of age, then on average you have lost about half your olfactory capacity.

Irv Rosenberg: Like so many age-related functional changes, we have to be careful because we tend to infer aging processes from cross-sectional studies. The cross-sectional data do show a decline, but there is an increasing heterogeneity within the population and some people have pathological processes which induce nasal epithelial changes, thereby limiting function. This is not, therefore, necessarily an age-related process.

Dan Storm: Interestingly, one of the primary clinical indications of the development of Parkinson's disease is the loss of olfaction.

Philip James: Dan Storm, you indicated yesterday that this also occurs in Alzheimer's—why should the same phenomenon occur in two such distinct pathophysiological conditions?

Gunnar Bergström: We are currently involved in a European project on the sensory capability of the European population, where it becomes clear that there is increasing variation in the olfactory capability in the population as they become older.

Ed Rolls: In Parkinson's disease, we have to recognize that there is a very marked dopamine neurotransmitter involvement in the orbito-frontal cortex so that when the dopamine system begins to deteriorate, you can expect olfaction and taste perceptions to decline. Interestingly enough, one of the features of fronto-temporal dementia is obesity and that is apparently caused by the induction of a grossly exaggerated preference for sweetness with the consumption of a lot of sweet drinks.

Gunnar Bergström: Is the decline in olfactory function a general phenomenon or is there a selective decrease in the response to particular compounds? Are we dealing with a general change in sensitivity from youth onwards or not?

Bo Angelin: Can we reverse the pattern of change in olfaction in old age? Furthermore, is there a correlation between the early loss of olfaction and premature aging, because this could be used to predict an important process?

John Kauer: I know that Dick Dotty has tested a small number of odors and found that the normal population shows remarkable heterogeneity in their sensitivity to particular odors. In the normal population there are a whole variety of anosmias as well as what we may call hyposmias, i.e. a reduction but without the absolute loss of specific odor sensitivity.

Barry Keverne: I suspect that the age-related decline reflects a combination of a failure to regenerate the receptor neurons as much as it is related to their selective loss. The stem cell population that produces the olfactory receptors presumably cannot go on indefinitely. I just wonder, therefore, whether, given in evolutionary terms, our protracted lives, with several decades of life after the end of the reproductive period there is no evolutionary drive to preserve such functions? It has also been known for years that olfactory sensitivity is altered by changes in motivation. So a hungry animal has many more neurons that respond in the olfactory bulb. Some years ago we worked on sheep and discovered that ewes acquire a specific olfactory recognition of their offspring soon after birth. Thus, electrophysiological recording in the olfactory bulb showed that only one or two cells responded to lamb odor and amniotic fluid, but 80% of the cells responded to food. However, after parturition, 80% of the neurons responded to the lamb odors, demonstrating an enormous increase in sensitivity. Microdialysis studies have revealed the release of gamma amino butyric acid and that this reflects neuronal activity. There is an enormous increase in this at birth and this then leads to a greater response at each subsequent birth, with lamb recognition taking much less time for the mother. Thus, there was a specific sensory period at birth which is

crucial for acquiring this sensitivity which can then be found a long time after lambing.

Stephan Rössner: I am in the schizophrenic position of both running an obesity clinic and being a member of the Royal Gastronomic Academy! I just wonder who should be cooking in the kitchen in old people's homes and seasoning the food? If you have young chefs doing this job, they are spicing food to their own satisfaction and perhaps not addressing the needs of the older people. Perhaps we need an appropriate reference person in the kitchens to tell the chefs what is truly needed by the older generation. Another aspect of this everyday problem is the fact that there are professionals who train to taste wines. Could we help perfect their training by giving them some medication to improve their learning capacity and, indeed, how do they cope when given drugs for their own illnesses?

Philip James: I have the impression that we should stop any chef from smoking because these cooks seem to load the food with salt implying different taste and odor sensitivities in smokers—is this true?

Gunnar Hall: A colleague recently presented her thesis on the age-related responses to food flavors and found that the elderly never complained that the food was too spicy, whereas the young often considered the food to be too highly spiced.

Solomon Katz: In evolutionary terms, since the acquisition of fire, we have found that it is women who prepare the food. Given the fact that niacin deficiency seems to be twice as common in women as in men, perhaps the women making the special peekie bread not only become sensitive to its color, but to the other flavor properties and thereby alter their food habits.

Stephan Rössner: It is amazing that almost all the top chefs in the world are men.

Barry Keverne: The issue of sensitivity to pheromones is complex. We know that the signals are mediated by the main olfactory tracts and that these are relayed to the piriform cortex. Yvanka Savic-Berglund showed very nicely this activity in the piriform cortex, but those individuals who were anosmic showed no activity. I know that there was also a response in the hypothalamus but we have to remember that the hypothalamus itself is packed with steroid receptors and if one is testing patients with steroids with inhalation experiments, then these steroids could very rapidly pass into the cerebrospinal fluid. Many years ago we studied the value of a new oral contraceptive by giving low doses on an inhalant stick inserted into the nose of monkeys. However fast we sampled the CSF after inhalation, the steroids were already there, so any hypothalamic response to odorants may actually involve routes other than olfaction. Obviously, if patients have clinical problems such as nasal polyps, then these will block the passage of molecules into the CSF as well as impairing the function of the olfactory receptors.

Stephan Rössner: It is a common observation that patients taking cortisone and analogous steroids increase their weight and this may involve overeating carbohydrates. We get a lot of referrals to our clinic where the steroids are needed for the underlying disease. What do we know about the way corticosteroids affect eating behavior, and why does it only seem to affect some patients?

Pheromones in Humans

Kjell Döving: I'd like to comment on the VNO. If you look for the VN cavity in humans one finds that the great majority of them do not have this cavity, so I would be very reluctant to accept that the VNO exists in humans. I just wonder whether Ivanka Savic-Berglund assessed the presence of this VNO by looking for the VN cavity in her subjects?

Ivanka Savic-Berglund: We did look for the VNO in our anosmic patients, but in our earlier studies on normal subjects we did not, as this was not an issue at the time.

John Blundell: I want to know how much human behavior is driven or influenced by pheromones.

John Kauer: The best example is that of Martha McLintock, who showed a synchronization of menstrual cycles in women who lived together. This seems to be mediated by an odor cue but whether this is pheromonal or not still seems uncertain.

Lars Hanson: I wonder about the basis for the selection of mates by olfaction. A mother's immune system reacts against the paternal antigens in the fetus and her cytokines are driving pregnancy. This involves a whole sequence of events from implantation to the growth of a placenta and affects the size of the newborn and the hormonal status. In Pakistan about 48% of the parents are cousins and amongst their newborn, 20% die more frequently than babies of non-related parents but the birthweights of the survivors are significantly less. Now I am told that HLA status of somebody determines their ability to smell, so is this smell system involved in the choice of mates? If you have cousins, the mother's immune system reacts less and the smaller baby is then more vulnerable.

Barry Keverne: I know of no good scientific evidence that human pheromones are important. One needs to be sure about the definition of pheromones, this having originally come from work on insects. In McLintock's work, the menstrual synchrony has not been shown to relate to a pheromone and may simply reflect some social or lifestyle input. Furthermore, it was not total synchrony, but simply a shift in menstrual timing. I think so much of human behavior is dependent on multiple sen-

sory cues, and we have heard from Ed Rolls how in man olfaction is dwarfed by the input of the visual system. I doubt whether any stereotyped behavior in humans is induced by pheromones.

Linda Buck: There is a popular misconception by those studying pheromones in humans that the vomeronasal organ is the only organ that senses pheromones, but I think there are many rodent studies that suggest that the pheromones are detected by this organ and/or the olfactory epithelium. There is no evidence that the removal of the VNO removes the pheromone response completely. In practice, the effect is rather subtle, whereas there is a dramatic effect in responsiveness when one removes the olfactory bulb which obtains input from both the VNO and the nose.

John Kauer: The best studies on pheromones in mammals relate to androsteindione in pigs. There is a cue to be found in a boar's saliva which governs the degree to which the sow moves into a lordotic position for mating and this also depends on whether she is in estrus or not. It has been shown that the VNO is not at all involved in this response.

Christer Löfstedt: I think we should stick to the original definition of a pheromone, i.e. a chemical signal emitted by an individual and received by another of the same species and which then elicits a behavioral or physical reaction. This definition is not restricted to sex pheromones and other behaviors and physiological reactions can occur. Most other mammals have been found to have pheromones, so I would be surprised if they do not occur in humans. There are many chemical signals that elicit stereotypic responses in some species. There are compounds that attract an insect or a mammal to feed on a particular plant, and mammal pheromones are widely used for marking the territorial limits of an animal. Chemical communication is rather ineffective at a distance if there is no wind. I think we should look for human pheromones but not restrict these to those involved in sexual responses.

Philip James: Solomon Katz, when you describe the diversity of cultural patterns and set out your understanding that the liming of maize to avoid pellagra was a learned, societal feature, you did not mention whether taste and odor might be associated with the liming process and the changes thereby induced. Could humans have been influenced by the smell of the process and its physiological responses—you talked about biscuits and bread in relation to smells, so could this not be an important part of the learning process?

Solomon Katz: Among the Hopi I emphasized the mate-selection process—it is very proscribed to whom the women can propose, so no issue of taste or smell could be involved. I have always suspected that the particular source of the lime may relate to the ash content and that trace elements within the ash, for example, zinc, may alter taste sensitivity. So if you make the peekie bread on a metal pot instead of a stone, it apparently tastes completely wrong and no one will want to eat it. There are a lot of subtleties to this and it would be useful to know what sensory processes are involved.

James Stubbs: I guess that in assessing pheromones in humans we ought to consider the issue of dominance because we are an intensely social species, influencing other people. So the question is whether, in primates, pheromones are important and provide any role in the establishment of dominance within a group.

Barry Keverne: Many years ago I worked with monkeys and how females communicate their attractiveness to males. We discovered that males learned of the attractiveness of females through odor cues, but once these monkeys moved into a social group, then the odor cues were completely dwarfed by other factors. Thus, you could alter the odor of a female to make her much more attractive, but it had absolutely no effect whatsoever when one tested the pairs of monkeys, so social factors become far more dominant and regulate behavior.

Cutberto Garza: Finally, I would like to highlight the role of leptin in the regulation of taste receptors where they were up-regulated when the leptin levels fell. This might then relate to a fundamental mechanism in energy regulation.

Albertino Bigiani: I know that leptin affects the potassium channels and taste receptor cells and enhances their conduction so the cells alter their excitability, but I do not know if these cells express sweet receptors.

Philip James: We have had an extraordinary tour of the diversity of responses in taste and olfaction, how these differ between species, and the remarkable way in which early, critical events in fetal life can imprint permanent changes which alter the sensitivity of receptors and their neural processing. The later behavioral programming also relates to the rate of receptor turnover with remarkable effects operating over longer time scales that then take us into the social and cultural dimensions of human adaptation. It has become evident that we are entering a fascinating stage in our understanding of taste and odorant sensitivity. If we are already at the state where the extraordinary array of changing foods minimizes primeval regulatory systems relating to the control of energy intake, then we may indeed have moved into a new phase in evolutionary terms. The early imprinting as well as the later adaptive programming of salt sensitivity is also of importance to public health, so we can now see that the detailed studies of the molecular processing and cell regulation of our taste and odorant receptors are indeed not only fascinating in molecular and cellular terms, but of great physiological and perhaps, indeed, of public health significance.