Ann Natl Acad Med Sci (India), **34(1):** 1-2, 1998

Editorial

It is with great sorrow and a profound sense of loss that we at NAMS share our grief at the passing away of Emeritus Professor Dr. Baldev Singh, Founder Fellow of the Academy and academician par excellence. Prof. Baldev Singh had character and merit, qualities of head and heart which endeared him to generations of physicians, neurologists, physiologists and medical teachers. He possessed the rare blend and balance of knowledge with humility, professionalism with perseverance and erudition with earnestness. His life and living was a shining example of an ideal physician committed to advancement in knowledge in basic as well as clinical sciences. Every one who came in contact with his towering personality and depth of demeanour felt overwhelmed and enriched. To honour his contributions to medical science and medical education, we dedicate this issue to his memory so that we may continue to reflect with gratitude on his contributions and seek continued inspiration from his life and laurels.

J.N. Pande

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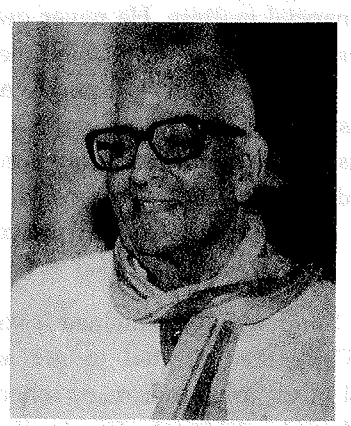
A personal homage

Emeritus Prof. Baldev Singh



(1903 - 1908)

When he died at the age of 95 years, Prof. Baldev Singh had already become a legend in his own life time. It is inconceivable to imagine a historical narrative of the 20th century Indian neurology without Baldev Singh emerging as one of the truly great professors of neurology. Indeed, it not only applies to India but rather to the whole Indian sub-continent where he was fondly called the 'Father Neurone', only to be replaced later by the more reverential 'Grandfather Neurone'. For nearly half a century, he strode across the country as an intellectual giant, generating awe and admiration amongst generations of medical students, residents and more importantly fellow neurologists and neurosurgeons. Whether on the bed-side, or in any one of the investigative and research laboratories devoted to electrophysiology, experimental neurology, tissue culture or neurochemistry, Baldev Singh left an indelible mark of his personality both as an academician, and as a human being with warmth and compassion for his patients, colleagues and students.

Many of us remember him, and shall continue to do so, with ever-lasting affection and immense gratitude for his teaching, his farsightedness, his common sense, his humour, his zest for life and living, and above all, his ability to be at ease, equally, with the highest (Jawaharlal Nehru was one of his admiring patients following the stroke that he suffered at Bhubaneshwar in January, 1964), and the lowest (even the lowest ranking employee of the

department of Physiology, AIIMS could share his or her personal burden with him and seek his help and assistance). Of course, as a human being he had some frailties-but these were exceptional. The generosity of his heart contrasted in an extraordinary manner with the thrift in his personal life and caution even in small financial matters. He never owned any vehicle during all of the nearly forty years that he spent in Delhi; there is an unsubstantiated but almost certainly a true anecdote, although not without subsequent embellishment, about his being found travelling in a DTC bus when wireless messages were being flashed all around to locate him because the then Prime Minister of India needed him for health reasons. In any case, all this added to the aura of romance and fascination which he continued to generate all through his life.

In his heart, Baldev Singh always remained a student with an insatiable quest for knowledge. Once the narrated to me an incident of his early childhood: he had failed in the annual primary school examination. Weeping bitterly, he told his mother about his intention to discontinue his studies and devote time to farming. His mother, either out of belief or tradition, consulted the village astrologer for 'vocational guidance'. Dr. Baldev Singh recalled with a chuckle; the astrologer told his mother 'the young boy will never leave study of books and even at the time of his death, few books will be found under his pillow'. Indeed, in the last five years of his life when he was visually handicapped, it was the duty of his doting daughter-in-law, Indu, to read to him the latest advances in neurology from the National and International journals that he continued to subscribe, even after donating his entire library to the AIIMS.

Although customary, but in the case of Dr. Baldev Singh the least necessary, a few important milestones of his life may be mentioned. He was born in village Gandasingh Wala (named after his grandfather who served in the sikh army) of district Amritsar on April 13, 1903. As he recalled years later in an intimate personal conversation, it was a most eventful day recorded in the family archives; he was born, his elder brother got married, and his grandfather Sardar Ganda Singh died on the same day within a span of a few hours!

After his early education in the village school, he did his F.Sc. (Medical) from DAV College, Lahore, securing the top rank. Admitted to King Edward Medical College, Lahore, Baldev Singh graduated in Medicine in 1927, with several medals and prizes. He proceeded to England in 1929, qualified

MRCP (London) in 1930, and was inspired by the great clinical teachers of that era. The complexity of central nervous system had always excited him since his student days. He enrolled himself at the National hospital at Queen Square which had already built a formidable reputation, thanks to the genius of Brown-Sequard, one of the original members of the staff at the time of the opening of the hospital in 1860, Hughlings Jackson (who died in 1911 but was immortalised as Plato of neurology) and Sir William Gowers. Although all three had died long before Baldev Singh entered the hallowed portals of Queen Square, he immediately 'came under their spell' as if 'they were still there, beaconing and encouraging, quizzing and demanding'. In particular, it was the marble bust of Hughlings Jackson which always 'transfixed his gaze at me'. Perhaps, this subconsciously initiated Dr. Baldev Singh's interest in epilepsy. Of these who were consultants at this hospital in 1929-31, Dr. Baldev Singh had nostalgic memories of the lecture demonstrations of Macdonald Critchley, although he was much more enthused by Derek Denny-Brown who was then an Assistant Physician at Queen Square but spent much time in the Department of Physiology at Oxford. Perhaps this combination of practice of clinical neurology and research in neurophysiology impressed the young mind of Baldev Singh and the image of Denny-Brown blending with ease the two assignments became etched in his memory and served as a role model in the later years of his life. It was also around this time, that the first paper describing electroencephalography in the humans had been published by Hans Berger in 1929, and in 1932, Nobel prize in Physiology and Medicine was awarded jointly to Charles Scott Sherrington and Edgar Douglas Adrian for their studies on 'Function of Neurones'.

It was Sherrington, the British neurophysiologist, who had coined the term synapse to describe the functional gaps between neurones and muscles and between individual neurones. Indeed within a short span of the preceding two decades, Pavlov, Golgi, Ramon Y Cajal and Sherrington had won Nobel prizes unravelling the mysteries of neurones and how they 'talk to each other'. Baldev Singh had sat 'mesmerized' when Sherrington delivered a talk at the Royal Society in London. A combination of these events perhaps set the course of his life's academic sojourn in neurosciences. He returned to India to practice academic medicine at Amritsar, but maintained his interest in neurology through an honorary attachment to the local Medical college. With his growing fascination for neurosciences, Baldev Singh decided to discontinue his lucrative practice and proceeded to the USA in 1950 to learn

electroencephalography and for further training in neurology. The rest is history.

With a neurosurgeon, Dr. Jacob Chandy, he laid strong foundations of Neurosciences at the Christian Medical College, Vellore, (1951-54) returning to New Delhi in 1954 as the Consultant Neurologist at the Tirath Ram Shah Hospital with clinical attachments for teaching and research at Lady Hardinge Medical College whose Principal at that time was Col (later, Major General) Amir Chand, an old teacher of Baldev Singh from Lahore days; His association with the Lady Hardinge also initiated a collaboration with the Department of Physiology where a young Professor, Dr. Bal Krishan Anand, had taken over as the Chairman. The initial collaboration matured into a life-long friendship and launched Bal and Baldev and Gulzar Chhina, on a voyage of neurodiscovery.

On a personal request by Prime Minister Nehru, and with a most persuasive effort by the then Director, AIIMS, Dr. K.L. Wig, Baldev Singh accepted the foundation Professorship of Neurology at the AIIMS in 1964, at the 'ripe' age of 61 years (which was already beyond the prescribed age of retirement). His reputation attracted amongst others, a youthful neurosurgeon, Prakash Tandon, to the AIIMS where Dr. Baldev Singh nurtured his young talent with care and concern. A DM course in Neurology and an MCh. course in Neurosurgery were soon started. Following his formal retirement in 1967, Dr. Baldev Singh served as Emeritus Scientist, ICMR and later as Emeritus Professor, with conjoint assignments in the departments of Physiology and Neurology. The death of his only son, Birinder (who was a Professor of Radiology at Medical College, Amritsar) for once shattered his equanimity and old age suddenly began to grow on him. With rare courage and fortitude, he continued to serve the AIIMS till 1991, when he finally left Delhi to spend the autumnal years of his life in the ancestral house in his village near Amritsar.

Several times, after visiting him in the serene environment of his study-cum-bedroom in the ground floor of the ancestral 'haveli' which like him, had seen better days and better times, one came away somewhat sad and depressed seeing his failing health, although he always made a special effort to compensate it by his soaring spirit, booming voice, and above all, an infectious enthusiasm. Nevertheless, it was clear that the once great intellectual 'luxury liner' was gradually sinking. The end finally came, after a short

illness, on February 2, 1998 when he departed from this mortal world, leaving behind two daughters, Tripta and Sudesh, a daughter-in-law, Indu, several grand and great grand children and a large circle of friends, admirers and students, some of whom having already become 'great' in their own right.

From amongst such a galaxy of scientists, constituted by his students and younger colleagues whom he nurtured with care and devotion, only a few were present at his last rites in his village. Nevertheless, it was not difficult to discern amongst them the reflected glow of their instructor and mentor, Dr. Baldev Singh. Issac Newton once said of himself, "If I have seen further than other men, it is because I have stood upon the shoulders of giants". Indeed, there are several in the country today who became great only by standing on the shoulders of Dr. Baldev Singh. Alas! most of them were not present when the last prayers for the departed soul were said on February 5, in the simple and dignified ceremony at his village, with the backdrop of the rays of setting sun providing a befitting aura to the chant of Vedic hymns.

Unlike some of those scientists of today who opt to become more competitive in their gush for individual rewards and glory, Dr. Baldev Singh all through his life had a singular devotion to, and gust for, knowledge and truth. He may not have made vital new discoveries but he certainly never failed to recognise the rare spark amongst some of the eager young minds assembled around him, and ensured the growth and development of their full intellectual potential. The crowning glory of Dr. Baldev Singh's odyssey through the exciting biomedical advances of the twentieth century does not lie in more than 250 scientific papers or numerous chapters in monographs and text books that he published; it lies in the simple fact that Dr. Baldev Singh knew how to recognise and nurture the talents of others, and admirably succeeded in doing so - always.

Throughout life, it was always that he honoured awards rather than the awards honoured him. The President of India decorated him with the coveted 'Padma Bhushan' for his outstanding contributions. The rank of Honorary Brigadier was conferred upon him by the AFMC which he served as senior Honorary Consultant Neurologist and Advisor for research in neurosciences. He was a Founder Fellow of the National Academy of Medical Sciences, and a Fellow of the National Science Academy. He was the reluctant recipient of

several academic distinctions, medals and awards but could never be persuaded to accept any elected office of a professional association. His scientific contributions are being commemorated in this special number of the Annals of NAMS.

Once during an intimate conversation, I asked him what epitaph he would prefer for commemorating his life's contributions. A lover of Urdu poetry as he was, he recited the following couplet:

'Bade shauq se sun raha tha zamana
Hum hi so gaye dastan kehate kehate'

'It was with great enthusiasm
that the world stood enthralled
listening to my discourse: it is only
I who went to eternal sleep while a
part of my narration was still unsaid'.

Yes, Sir! you were so prophetic
even in the choice of your own epitaph!

J.S. Bajaj

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Dr. Baldev Singh (1903-1998)
Reminiscenes and Personal Vignettes

Dr. Baldev Singh, the founder fellow of the National Academy of Medical Sciences, was born on 13th April 1903, at Zaffarwal Dutta (now in Pakistan), in a family of aristocratic rich landlords. His elder brother persuaded him to choose medicine as his career. He did his graduation from King Edward Medical College, Lahore in 1927 with honours. He got married in 1928 and then left for U.K. where he obtained the Membership of the Royal College of Physicians in 1930.

After returning to India, he started his general practice in his village near Amritsar in 1931. Within a few years, he developed a lucrative practice and lived an aristocratic life. Despite this luxurious lifestyle he was deeply committed to academics. The urge to learn was so deep in him that he decided to study further at the age of 45. He wound up his practice and left for Chicago, towards the end of 1949, to learn electroencephalography from Prof. F.A. Gibbs. On his return to India in 1951, he joined as Associate Professor in Neurology at Vellore. At the same time, Dr. Chandy was working as a neurosurgeon in the same institute. In a short time, as a result of the combined efforts of Dr. Chandy and Dr. Baldev Singh, Vellore became the main centre of clinical neuroscience in India.

In 1954, Dr. Baldev Singh was invited to join Tirath Ram Shah Hospital in Delhi. Never satisfied with patient care alone, he also started his research in collaboration with Dr. B.K. Anand, who was then heading the Physiology Department at Lady Hardinge Medical College. This fruitful association continued even after both of them shifted to the All India Institute of Medical Sciences (AIIMS). In 1964, Dr. Baldev Singh was invited to establish the Department of Neurology at AIIMS. In the three years that he was in active service at the AIIMS, he managed to establish a good Department of Neurology. Even after his retirement, he played a role, behind the scene, in the establishment of the Neurosciences Centre at AIIMS. After his retirement from the department of Neurology, Dr. Baldev Singh joined as an Emeritus Professor in the Department of Physiology at AIIMS. It is very rare for a professor of a clinical discipline to opt for a position in a basic science department. In the Physiology department, he had his room next to that of Dr. G.S. Chhina, with whom he was closely associated in most of his research work. There he conducted research in areas like high altitude, yoga, biofeedback, sleep, consciousness, cerebral oedema, epilepsy and heat hyperpyrexia. During that period, collaborative research and teaching by the Department of Physiology, and clinical departments, became the order of the day.

Many seminars in the department of Physiology were moderated by the faculty members from clinical disciplines. All the seminars in the department of Physiology were attended by several postgraduates from clinical disciplines. The vertical integration in teaching and research in medical disciplines was actually practised without much hype and publicity.

During his stay in the department of Physiology, AIIMS, he actively participated in lectures and seminars. He always prepared his lectures very meticulously, and rehearsed them several times before the actual presentation. In his preparations, he never differentiated between an important oration and an undergraduate lecture. His personal library had books which were not available even in good medical institutions. He kept himself abreast with the latest developments in science, especially neuroscience. But he was never too eager to 'show off' his knowledge, and was never ashamed of admitting his ignorance. The tenacity with which he followed the problems was a good example for the students of follow.

Dr. Baldev Singh was awarded the Padma Bhushan in 1971 by the President of India. He was a recipient of several awards and recognitions incluidng the Basanti Devi Amirchand Award, Air Marshal Subroto Mukherjee Award, Sir Nilratan Sircar Oration, the National Academy of Medical Sciences Oration, and Chandy Oration. In addition to being a Founder Fellow of the National Academy of Medical Sciences, he was also the Honorary Fellow of Aeromedical Society of India and the Indian National Science Academy. He was also an Honorary Brigadier of Indian Army.

More than his research and other achievements, his life itself was an inspiration to his friends, colleagues and students. He led a simple life, and had his meals at the students hostel at AIIMS. He was a highly cultured person, with an unquenchable thirst for knowledge. He had a deep love for poetry and philosophy. At any function, one could count on him for an apt Urdu couplet. He maintained a low profile in public life, and had virtually no social life.

He never tolerated injustice and dishonesty around him. He was known as a "no-nonsense man" and never hesitated in calling a spade a "spade". He believed that honesty is an essential trait, and that a person who is not honest in personal life cannot be honest in science too. He shunned praise, publicity and popularity. He was visibly angry when the young postgraduates of the department of Physiology once decided to celebrate the birthday of their beloved "grandfather neuron", as he was popularly referred to. He had always shown deep concern for the younger members of the department. He used to reach the department before anyone, and leave the place by late in the evening, when it was time for his dinner. Before leaving the department, he would go around and say "good night" to the young postgraduates

who would be working. For him, science was not something which was just confined to the laboratory and books. The human being who pursues science is as important as science itself. He had always played a major role in diffusing tension amicably and in building good relationships between scientists, students and teachers. The sincerity with which he approached the problems, always convinced everyone concerned. The department of Physiology, at AIIMS owes a lot to him for this contribution too. His failing eyesight compelled him to leave AIIMS in 1992. He spent the last six years of his life at Amritsar under the affectionate care of his daughter-in-law, Indu, who also catered to his first love, academics, by reading out to him. She had also appointed one peson to read out to him from newspapers and magazines.

Dr. Baldev Singh, left for his heavenly abode on 2 February 1998 after a brief illness. I would like to end this note with a personal vignette. My most memorable encounter with him happened a few days before his sad demise at his home in Amritsar. He lives in a traditional aristocratic home, which could be best described as a "Haweli". He was totally blind, and it was a shocking sight for me. I was finding it difficult even to greet him. But he laughed and talked about everyone in the department and the institute. I was surprised to find that he had kept himself abreast not only with the happenings in the scientific world but also with everything concerning the individuals with whom he had been associated. After spending about an hour with him, I had to take leave. I was overwhelmed and finding it difficult to say "bye" to him, but he, on the other hand, showed no emotion. He was all smiles and enthusiasm. He had no fear, no hesitation and no sorrow to express. On that day, I learned some very important lessons in life. Dr. Baldev Singh will ever remain a source of inspiration to a very large number of his admirers, friends and students.

May his soul rest in peace !

Dr. V Mohan Kumar
Professor of Physiology,
AIIMS, New Delhi
Fellow of NAMS

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Dr. Baldev Singh, left for his heavenly abode on 3 February 1998 after a brief illness. I would like to end the note with a personal vignette. My most memorable encounter with him happened a few days before his sad demise at his house in Amritsar. He lives in a traditional aristocratic home, which could be best described as a "Haveli". He was totally blind, and it was a shocking sight for me. I was finding it difficult even to greet him, but he laughed and talked about everyone in the department and the institute. I was surprised to find that he had kept himself abreast not only with the happenings in the department but also with everything concerning the individuals with whom he had been associated. After spending about an hour with him, I had to take leave. I was overwhelmed and finding it difficult to say "bye" to him, but he, on the other hand, showed no emotion. He was all smiles and enthusiasm. He had no fear, no hesitation and no sorrow to express. On that day, I learned some very important lessons in life. Dr. Baldev Singh will ever remain a source of inspiration to a very large number of his colleagues, friends and students.

May his soul rest in peace !

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Neurobiology of Feeding Behaviour : A Perspective of Last Four Decades*

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Feeding behaviour is regulated by a multiple-sensor, closed-feedback system, with regulated input as well as output. The driving biological need, linked to the internal physiological deficits or biologically relevant events in the external envionment is reflected in the changes in different major homeostatic indices determining the normal metabolism, and interact with the environmental factors to form the basis of the biological motivation. There is a distinct relationship between sensations (e.g. hunger) and drives (e.g. food intake). Stimuli (internal or external) adequate to elicit a sensation also elicit activities directed towards reducing the intensity of such sensations, i.e. the stimuli for those sensations induce motivational states that drive the organism, to provide whatever is felt to be lacking. Viewed in this respect, chemosensory signals from food provide the sensory basis of hedonic matrix that controls food acceptance, choice and intake (1). The interfacing of these signals with the changing needs of the organism, include the concept of priorities, competition and compromises in regulation of several factors contributing to homeostasis (2). The fact that an identical stimulus may be handled differently by the nervous system depending upon the variety and complexity of existing variables or the capacity of the nervous system to make continuous appraisals and instant decisions in order that the organism can react in strict accordance with the requirements of internal and external environment, is all well known. Aspects of this inherent plasticity in the nervous system are described below with electro-physiological and behavioural evidence in the domain of feeding behaviour.

Early Studies

The classical paper of Anand and Brobeck (3) showed that tiny discrete lesions of specific, localised zones in hypothalamus bring about aphagia on the one hand, and hyperphagia and obesity

* The present article is our acknowledgement and a modest tribute to the memory of Dr. Baldev Singh, whose inspiring guidance at every step of our research and personal attainments has served as a beacon light since the early fifties.

on the other, depending on the site of the lesion. The main stream of thought during the fifties placed both detection and control of food intake regulation in central nervous structures in which hypothalamus plays a major and decisive role (4). It was gradually realised, however, that though these central nervous structures may play an important part in regulation of feeding behaviour, a number of fundamental questions remain unanswered and point, inter alia, to the existence of peripheral receptor systems involved in food intake and extend well beyond the classic sense of taste and olfaction; and this formed the major thrust of the research activities during the sixties and the seventies.

Alimentary Receptor Systems

Gastric distension, activating distension receptors and simulating a fed state, evoke gastric afferent activity and influence hypothalamic "satiety" and "feeding" centres, the activities bearing an inverse relationship (5). Perfusion of stomach is also shown to activate gastric afferents, and influence selectively localised nervous structures implicated in food intake (6). Probit analysis of response characteristics of the parallel neurons in brain-stem to gastric chemoceptive projections indicates that in addition to 'across-neuron pattern', spatio-temporal cues play an important role in conveying the quality message, while overall height of this pattern seems to indicate the intensity of the test material (7). Further, Sharma and Nasset (8) showed that intestinal perfusion with glucose and aminoacids produced relatively specific features of evoked response in mesenteric nerves, the central projections of which have been localised in hypothalamic regions. Results of this type indicate the existence of peripheral receptor systems in the gut (9), which possibly send information about metabolites in stomach, intestine or portal vein to central nervous structures (10). It is a fast-acting system detecting ingested metabolites and raises serious doubts about detection and control being a central phenomena.

We also know that alimentation requires both instant decisions and continuous appraisal of external elements serving as sensory stimuli for acceptance or rejection of food (e.g. palatability, social context, learned behaviour) and contrasts these factors with internal features such as gastric filling, blood sugar level and the metabolic or "energy pool" of the organism. Once a meal is initiated, its sustenance and termination are linked to the signals that act to monitor subsequent intake, increasing it in the positive feedback loop, or decreasing it in a negative feedback loop (11).

Initially, the oral sensory appraisal of food leads to its acceptance or rejection and, when accepted, is eaten in definite amounts. "This sensory activity to foods is a critical determinant of innate or acquired feeding responses, ensuring an oral selection and a metering of intakes. Through the second step of action of foods in feeding process, these orally determined responses to food are "regulated". At the post absorptive and systemic level, food as a nutrient acts as a metabolic signal on regulatory centres and 'modulates' oral

feeding responses" (12). It could be further suggested that sensory signals not only become important in controlling intake, but feedback into the efferent system controlling the energy pool, producing some of the metabolic changes ordinarily controlled biochemically at cellular level. In common sense terms, it could be hypothesized that the hungry animal eats for taste, and that the taste cues accompanying ingestion directly initiate some of the satiety signals (e.g. hyperglycaemia) which classically follow the normal process of digestion. Thus satiety cues are produced in two phases, first as anticipatory reflex initiated by the taste of food, and secondly by the postabsorptive metabolic events.

The role of gut afferents in ingestion and the possible mechanisms involved have shown a number of features that could be explained on the above basis. We know that administration of endogenous opioids like B-endorphin or encephalin stimulates both feeding and drinking (13) while naloxone, the morphine antagonist, suppresses food and water intake (14, 15), and influences differentially the intake of sweet and sapid substances. The significant reduction in intake of various solutions brought about by naloxone, is observed in animals with intact vagus but not in gastric-vagotomised ones, suggesting the possible involvement of endogenous opioid mechanisms in the gastro-gustatory interactions in taste (16). As peripheral cholinergic blocking does not seem to affect the taste behaviour of the animals, it is quite likely that the changes seen in intakes of sweet and salt solutions in vagotomised animals are predominantly due to the loss of vagal afferents from the stomach (17). The visceral vagal and gustatory afferents, first synapsing in tractus solitarius, project to parabrachial nucleus of pons, and thence to the hypothalamus, amygdala, bed nucleus of striaterminalis and also to the reticular formation. The interactions could be taking place anywhere in these sites. The probability of visceral afferents contributing to the control of ingestive behaviour via the 'solitary-reticular ingestion system' has been suggested, among others, by Norgren (18). The interception of these ingestive signals from the gut could be acting through the gastro-gustatory interaction sites mentioned above and conceivably bring about alteration in the gustatory preferences of the animals.

The flow of information from the alimentary receptors to the brain is not all in one direction but is rather achieved by 'turning' of the receptor systems through use of centrifugal controls. These controls allow sensory pathways to act as variable filters so that stimuli tagged with a particular attribute or feature are alone allowed through for detailed analysis. By such means it is possible to attenuate or amplify afferent signals, or switch on or off the inputs, thereby selecting a particular input at a particular time. These studies have shown that gastric and intestinal sensory mechanisms, operating at the intermediate level between oral and systemic factors, are concerned with the 'sensory' appraisal of food including its texture, viscosity, volume, temperature, and other physicochemical properties of diet. The gastrointestinal mechanism is a fast-acting system and shares in large measure the organisational control characteristics of the oral sensory system (19, 20).

What are the consequences of food ingestion? How does an organism being fed ablib or under conditions of food deprivation, or in varying states of hunger, handle the information from the dietary source? What signals operate before birth when feeding is aquatic and continuous? How are these signals related to the conditions in the adult, where assimilation within the body is also aquatic and continuous? How does the state of 'energy homeokinetics'-surfeit or deficit state, interface with external dietary and environmental cues to guide the feeding behaviour? How indeed feeding fits in the domain described under the rubric of homeostatic motivations? These and allied questions have attracted the attention of several workers during the last few decades. In the following description while global perspective has been kept in mind, work conducted in India has been highlighted and to that extent may show some bias.

Ontogeny of Feeding

Examples of ontogenetic analysis suggest that each stage of development is complete. The common supposition that adult regulations are in some way superior to those of the infant cannot be sustained. Physiological changes do more than keep an animal alive in a constant state, and, when repeated day after day, they alter as time passes and produce the long-term changes of ontogeny. Food and water affects, and is affected by, this adaptability. Observations on ontogeny of saccharine preference in neonate rats clearly suggested that the apparent learning curves for saccharine, were infact maturation curves (11) and were linked to the maturation of gustatory system which is complete by about 14 days of neonatal life of rat (21). It appears that neonate is primarily dependent upon 'taste', rather than 'calories', a feature also seen in adults under certain conditions of nutritional stress, food deprivation, metabolic disorders and psycho-sociocultural overtones.

An alternative possibility has also been proposed. It has been found that the neonate rats ate enough atleast to double the average growth rate if competition for food was eliminated by limiting the litter size. This would indicate that the neonate is primarily dependent on taste cues, and fails to regulate 'calories'. The above results may appear contradictory to the observations in adult animals who are considered to eat for 'calories' (2) and generally regulate body weight over a period of time inspite of the day to day variations in input-output relationship. However, these dissimilarities are, at best, only superficial. Need-related changes in palatability and taste sensitivity are well known. Our approach has been to vary hunger by mealtime restriction, graded food deprivation, insulin or thyroxine injection. We then observed food intake and preference shifts in responses to 'liked' items e.g. fat, glucose or saccharine or 'disliked' items like cellulose, NaCl or quinine which were added to stock diet or put into solution. Normal adult rats and dogs, hypothalamic hyperphagic rats and neonate rats have been used as subjects (1).

Taste Vs. Calories

Studies extended to include other animals than rat and using different methods to vary taste and calories independently have generally confirmed the notion that the importance of taste and calories is related to the state of energy balance: hunger potentiating taste (Figure 1). Physicochemical information from the diet feeds into two detector systems which can respond to signals from taste or calories. It is not a dichotomous system but both sets of cues are acting all the time. Whether the nervous system makes use of either set of signals in monitoring further intake is a function of the state of energy balance. The 'energy pool' acts as a biasing system, assigning priority to taste when the animal is in deficit and to calories when it is in balance or surfeit. The terms used here as 'taste' and 'calories' are infact meant to mean the 'sensory' and the 'metabolic' properties of diet respectively (22). This hypothesis is similar to 'behavioural regulation' hypothesis of Richter, Katz's avidity theory, and Le Magnen's concept of 'primary response' in assuming that organic needs alter perceptual bias on an innate basis so that the animal seeks out and ingests the needed food on the basis of its sensory qualities. The fact that food

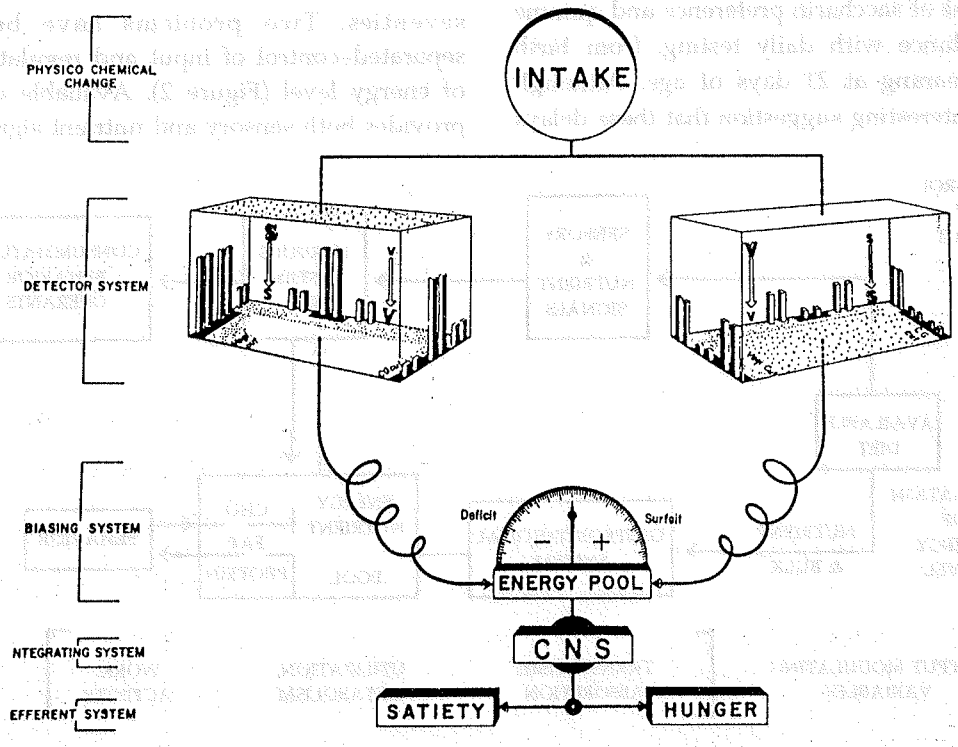

Figure 1. Model showing the dual detector system and the role of the energy pool as a biasing system, in the overall control of food intake (From Sharma et al. J Neural Transmission 33 : 113-154, 1972).

contains nutrients is considered coincidental.

For instance, adapted rats to chronic undernutrition when tested for oral ingestion of various solutions such as glucose, saccharin, sodium chloride, and quinine showed over-reaction of sweeter substances becoming more acceptable, and bitter substances becoming more aversive as a function of increasing degree of chronic food deprivation (23). This has parallels between such effects of food deprivation on gustatory responses and the gustatory responses observed during neonatal stages (11, 20). These results also indicate that there is a gradual development of saccharin preference and quinine avoidance with daily testing, from birth to weaning at 21 days of age. Although the interesting suggestion that these delays may be correlated with differential maturation of taste buds (necessary to discriminate the stimuli) cannot be overlooked, the results also suggest that if such acceptability for sweetness is reinforced, as partially happens with the lactose of mother's milk taken by neonates, this contiguous pairing confers a biological significance in establishing the sweet preference and in regulating intake on the basis of taste.

Control of Input and Regulation of Energy Level

There has been yet another very important dimension added as a result of the studies conducted in sixties and seventies. Two problems have been separated-control of input and regulation of energy level (Figure 2). Available diet provides both sensory and nutrient signals

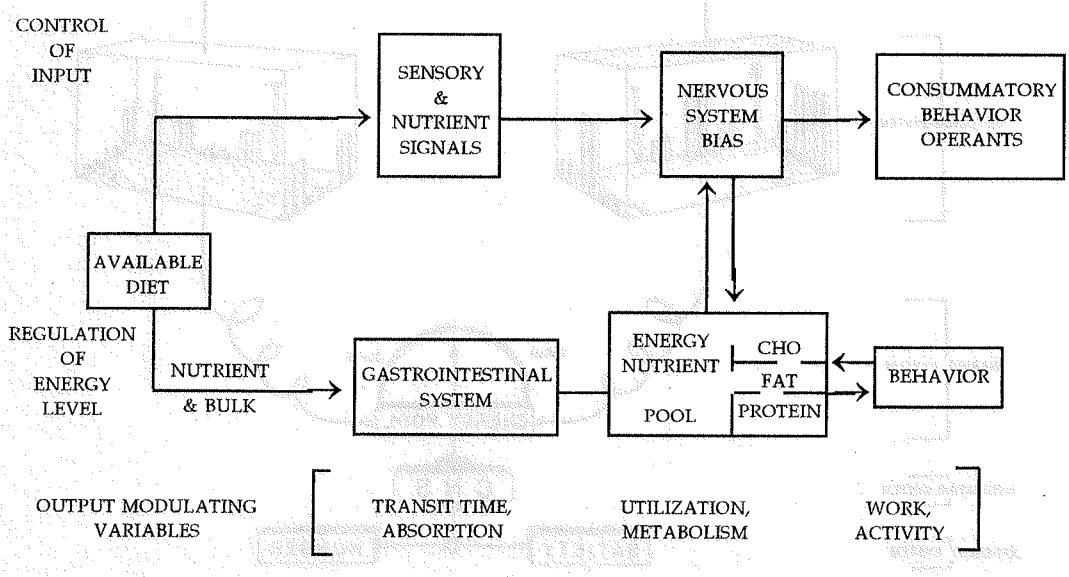

Figure 2. Scheme of input control *(top)* and energy regulation *(bottom)* as interacting systems involved in homeostatic regulation of consummatory behaviour (From : Sharma KN, In : Advances in Physiological Sciences, Macmillan India, 1992, pp 639-647).

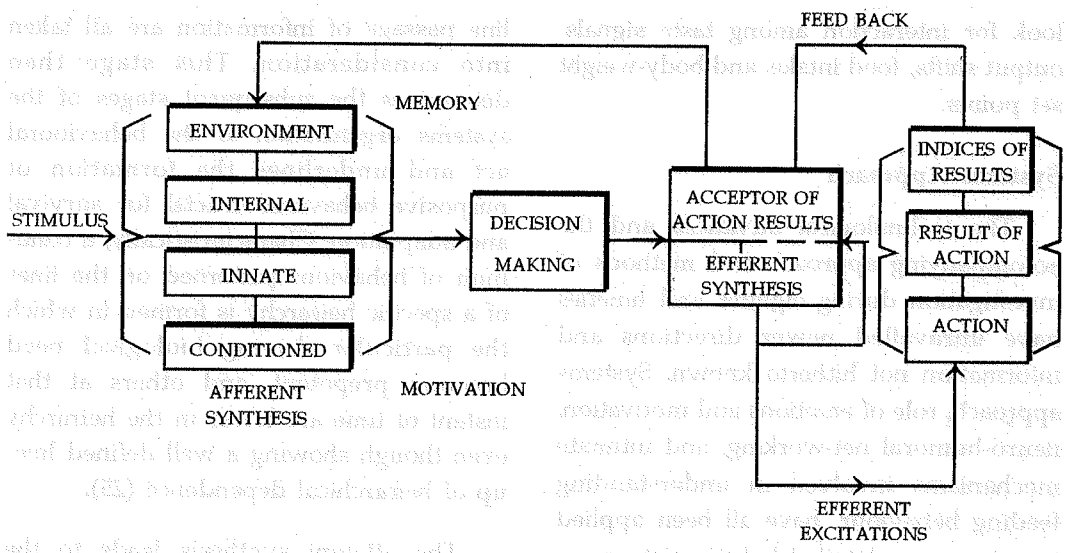

Figure 3. Schematic block diagram showing various interlinked and interacting stages depicting the relationship of stimulus characteristics, afferent synthesis, decision-making, efferent excitations, and the role of feedback control system and memory mechanisms in guiding the motivational behaviour (From : Sharma KN, In : Advances in Physiological Sciences, Macmillan India, 1992, pp 639-647).

that the brain in turn compares to other signals coming from the energy-nutrient pool, which provide information about the sensory and metabolic consequences of these nutrients. The brain then uses this information in modulating food intake (consummatory behaviour) or learned food related behaviour.

One finds that the available diet provides nutrients and bulk going to the body. From this point of view, the regulation of body weight can be effected by a series of output modulating variables, before, during, and after the food enters the energy-nutrient pool. It is easy to see that the combined action of gastric emptying time, intestinal absorption rate, efficiency of food utilisation and metabolic activity, and the behavioural output of muscular work and general activity can be important in regulating the energy-nutrient pool. As the energy pool is modified, it can influence feeding itself by providing appropriate signals as to its condition, which the nervous system uses in the control channel. The sensory signals from available diet and the two-way connecting pathways (vertical arrows) between the control channel and the regulating channel, provide a biological basis for the role of taste in energy exchange, as well as in food intake, by various possibilities of interaction between these channels. Thus in this scheme body weight is not only influenced by food intake and changes in output, but also by taste-induced appetite shifts from energy-nutrient pool (24). The scheme thus biases us to look at the regulation channel more directly than we have in the past and to

look for interaction among taste signals, output shifts, food intake, and body-weight set points.

Systems Approach

The technological advances and the accompanying approach and methods of investigation during eighties and nineties have unravelled newer directions and information not hitherto known. Systems approach, role of emotions and motivation, neuro-humoral net-working, and intimate mechanisms involved in understanding feeding behaviour, have all been applied to get a more detailed holistic picture.

Essentially, homeostatic motivations meet the driving biological needs in the preservation of the species or genus of the individual. The concept of motivation, according to some, is restricted to the analysis of behaviour that is goal directed, or need related, or purposive. Still others define the study of motivation as a search for the determinants of human and animal activity, ranging from systems approach to levels of membrane functioning and molecular and intracellular genetic memory. Each approach has its distinctive value, but is not divorced from the limitations it concurrently poses.

In this complex organisation of systems analysis (Figure 3) afferent synthesis forms the first step in which juxtaposition, selection and synthesizing of functionally diverse inputs linked to the dominant need, past experience or memory, simultaneous presence of other afferent stimuli, and straight through hot-line passage of information are all taken into consideration. This stage then determines the subsequent stages of the systems organisation of the behavioural act and underlines the formation of purposive behaviour crucial for survival and adaptation. Characteristically, a continuum of behaviour patterned on the lines of a specific heirarchy is formed in which the particular driving biological need becomes prepotent, and others at that instant of time are lower in the heirarchy even though showing a well defined line-up of heirarchical dependence (25).

The afferent synthesis leads to the specific state of readiness in which both motivation and environmental stimuli interact as activated by memory mechanisms and bring into focus the preparedness of the organism. This in turn leads to triggering of the behavioural response activation time and determining the whole act to include initial and subsequent stages of decision making, formation of mechanisms of predicting results, and satisfying the specific needs. Dovetailed with this is the next stage of decision-making in a manner that the behavioural act becomes imperative. It is precisely this stage that shapes the significance of resultant activity in need satisfaction, and form the basis of the organism's capability not only to monitor and correct behaviour errors but to bring about behavioural acts to their precise end point. The development of biological motivations appears related to a retrieval of genetic information stored in CNS cells, and can apparently induce such neuronal changes as lead to the activation of DNA and RNA fragments

responsible for synthesing specific polypeptides. This is the process which shapes the pattern of afferent synthesis and decision-making and with which the results of behaviour are constantly compared through feed back influences. Some of the recent findings have laid a fairly good groundwork indicating that the control and regulation of sensory and metabolic events resulting in the particular behavioural act, say, of feeding behaviour, may come from higher levels of the same sensory pathways, from motor pathways, from pathways mediating other modalities and a number of other sources. One of the ways by which this regulation is achieved is by the 'tuning' of receptors through the use of centrifugal controls via short and long feedback loops (22). It may not be difficult to surmise on the basis of such possibilities as to how centrifugal controls, by differently registering and responding to a particular stimulus or set of stimuli, could indeed influence the widely varying spectrum of feeding behaviour.

In a series of recent studies jointly undertaken with the Institute of Normal Physiology, Moscow, the above possibilities have been examined more closely. It has been known that neuronal population in lateral hypothalamus (LH) and the ventromedial hypothalamus (VMH), particularly the units responding to nutrient substance such as glucose, generally behave in a reciprocal manner, and the differential activity levels is linked to the state of hunger or satiety (23). This hypothalamic neuronal activity, influencing motivation of feeding and associated behaviour, can also be differentially influenced by the basal ganglia and frontal cortex supporting the concept of motivational systems guiding behaviour through such modulating influences. The reactivity of sensorimotor cortical (SMC) neurons to micro-iontophoretic application of oligopeptides such as tetragastrin and bradykinin in SMC neurons in hypothalamic-induced food reactions were investigated in unanaesthetised, freely-moving fed rabbits possessing chronically-implanted electrodes. The cortical neuronal activity was recorded in presence or absence of food placed infront of the animal following stimulation of lateral hypothalamus. The results indicate that lateral hypothalamic stimulation induced hunger and the animal would eat vigorously. In such a situation majority of the SMC neurons showed excitatory responses. If, however, LH stimulation was done but food was not placed in front of the animal, the same SMC neurons now predominantly showed an inhibition i.e., non-reinforcement of food, switched the excitatory pattern to inhibitory one. Micro-iontophoretic application of gastrin and bradykinin decreased the percentage of neurons showing activation on LH stimulation in the presence of food, but enhanced the number of positively responding SMC neurons in the absence of food. Thus gastrin and bradykinin produced qualitative changes in SMC neuronal responses to LH stimulation: neurons that initially responsed with activation or inhibition to LH stimulation changed their response in an opposite direction. It would seem that tetragastrin and bradykinin applications lead to specific reorganisation of the neuronal-circuits for

decision-making and theraby differentially influencing the motivational and reinforcing components in the composite feeding behaviour (26, 27).

Appetitive Behaviour in humans

What relevance does this type of behavioural and electrophysiological data in animals have to the observations in humans? The work of Thompson and Campbell (28) on human gustatory responses for both magnitude estimation and hedonic matrix shows a number of features obtainable from animal models. Their studies demonstrated increased hunger ratings as well as enhanced magnitude estimates of pleasantness for sucrose solution following infusion of 2-DG. The results are interpreted to show that 2-DG produces glucoprivation in neuronal units, as shown in experimental animals (29). The change in neuronal activity brings about variation in ingestive behaviour, favouring taste and showing the increased hunger state of the person, analogous to the food-deprived state in animals over-reacting to sensory properties of food (11, 30). The affective behaviour toward a sweet taste in man has been shown to be modified by a variety of changes influencing the internal state of the individual (31). For example, reduction in body weight increased food satisfaction in man and delayed ingestion-induced unpleasantness (30), much in the same manner as chronic food-deprivation in animals potentiated sweet taste. Similarly, insulin-induced hypoglycaemia increases a feeling of hunger in humans (32), which has parallel in insulin-induced gustatory and olfactory potentiation in animals (33). Perceived sensory intensity and hedonic value in neurons has shown important features of gustatory profile linked to internal state of the individual, and has a parallel in animal models (23). These studies thus indicate that nutritional background (internal state), previous dietary history (ontogeny of feeding), and external environmental factors interact in such a way that the prepotent sensory properties of food and experiential factors subserve to bring about relevant metabolic adaptive changes, taste preferences, and food habits.

When these studies were extended to high altitude conditions some interesting aspects have been revealed. Sojourners to high altitude (HA) often complain of anorexia leading to decrease in food intake, and the degree of anorexia seems to be related to the height of ascent. Sensory and metabolic signals from food are known to alter in certain nutritional stresses and one such stress is high altitude exposure, which causes decreases in food intake both in animals and man (1, 34, 35). This decreases in food intake in HA leads to decrease in body weight which is known to influence the food choice and uptake (36, 37). Our recent study (38) was designed to investigate the changes in taste responsiveness in terms of threshold, hedonicity and intensity in Indian soldiers during their stay at an altitude of 3500m. There was a change both in threshold and hedonicity. While threshold was increased, the hedonicity ratings for glucose at HA

were higher than at sea level, indicating increased pleasantness and palatability for glucose. Interviews with the soldiers at HA showed that while they had taken a full plate of food items, they did not feel like eating it after having a few bites. Perhaps it was the altered hedonicity which created this early satiation emanating from a "not-so-palatable" food. Sweet substances and carbohydrate-rich diet improved the appetite. It seems quite certain that once the nutritional stress leads to body weight loss or deficit energy state of the body, hedonics plays a rather prominent role in regulating food intake as observed at high altitude. Quinine sulphate and citric acid thresholds showed a decrease on exposure to HA, and appear to induce aversion at far lower concentrations than could be expected at sea level. This nicely fits with the bimodal model suggested by Jacobs and Sharma (11). The present study further indicates that providing a more palatable carbohydrate-rich diet could to a certain extent, ameliorate anorexia and body weight loss resulting from HA stress.

In conclusion it may be surmised that whether the sensory or the metabolic cues become prepotent to guide the motivated behaviour towards need reduction or homeostatic regulation of feeding, is a dynamic process in which both sets of cues are involved, and it is the interaction of internal milieu with the external environmental factors impinging upon the innate and experiential correlates which appear to determine the degree and the direction of the behavioural act. Thus emotional stress and motivational states can bring about not only a quantitative but a qualitative change in these need related determinants and consequently are expressed in overt forms of behaviour.

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Neural Regulation of Sleep as Understood During Pre-Baldev Singh and Baldev Singh Eras

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SUMMARY

Neurons in the midbrain reticular formation, primarily making up the ascending reticular activating system (ARAS), are important for production of low-voltage, fast-frequency, EEG pattern, commonly associated with wakefulness. The old notion that sleep is a passive phenomenon resulting from inactivation of the ARAS, is not true. There are specific brain regions that promote slow wave sleep (SWS) and others that are responsible for rapid eye movement (REM) sleep. Brain regions above the brain stem, namely the preoptic area (POA) of the hypothalamus, play an important role in the regulation of sleep. In spite of growing evidence in favour of the theory that the hypothalamus is the major centre regulating SWS, sleep-wake cycle, and even wakefulness, the recent findings confirm the important role of the brain stem in genesis of REM sleep. The importance which was previously given to the thalamus, for the regulation of SWS, is now seriously questioned, although it is still considered important for the genesis of the EEG spindles of normal sleep.

Chemical stimulation and neurotoxic lesion studies showed that the major function of the POA is sleep maintenance, rather than sleep initiation. Though there is interaction between thermoregulation and sleep regulation, there are different sets of neurons in the POA, endowed with the ability to regulate these two functions independently. At the same time, the POA could play an important role not only in integrating these two functions, but also in fine tuning the entire energy balance of the organism.

Key words: Sleep, Wakefulness, Preoptic area, Hypothalamus, REM sleep, Slow wave sleep, Brain stem, Thalamus, Ascending reticular activating system, EEG .

INTRODUCTION

This article is dedicated to the memory of Prof. Baldev Singh who had a deep interest in the field of sleep and consciousness. He had with him several books on sleep which were not available even in our libraries. Buying books was some kind of hobby for him. He used to lend those books to me, and discuss with me the theories of sleep prevalent during

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that period. Neurophysiology of sleep was a pet topic for him, and he used to select this subject, whenever he was invited to deliver lectures. The passion with which he spoke about this topic created such an interest in me that I took up this field of research and I have never deviated from it since then.

The understanding of neural regulation of sleep had undergone a drastic change in the last few decades. According to the classical concept of sleep which was prevalent till the sixties, which could be termed here as Pre-Baldev Singh Era, the neurons of the brain stem reticular formation played a major role in waking, consciousness and sleep. The later section of this article deals with the hypothalamic regulation of sleep, which dominated the scientific thinking during the Baldev Singh Era. The concept of sleep regulation by the brain stem, along with the latest modifications to the classical concepts, are also described here.

Brain stem regulation of wakefulness and passive deafferentation theory of sleep

Till around 1970, the sleep theory which dominated the text books were based on the findings of Moruzzi and Magoun (1). According to their findings, interruption of ascending flow of reticular impulses from from the brain stem (as it happens in the case of acute cerveau isole preparation), would result in a state of EEG recording which is similar to that of slow wave sleep (1). So, they concluded that the ascending influence, exerted by the brain stem on waking structures of the cerebrum, maintains the wakefulness. The sudden withdrawal of the influence of the ascending reticular activating system (ARAS) would be the cause of slow wave EEG pattern obtained during sleep. It was then shown that the EEG pattern observed in acute cerveau isole (isolated brain) preparation is irreversible, similar to that found in a state of coma; rather than that of slow wave sleep (SWS). On the other hand, the EEG pattern observed in SWS is reversible. So, the original theory of withdrawal of ARAS influence was modified.

According to a new version of the old theory of passive deafferentation, a tonic ascending flow of reticular impulses would be responsible for the state of wakefulness, its complete interruption would lead to a state of coma. But, a temporary slackening of the tonic ARAS discharge might be responsible for the onset and the maintenance of natural sleep (2,3,4). These doctrines postulated that sleep is basically an inability to remain awake. When the tonic barrage falls below a critical level, the animal would fall asleep, simply because it is unable to stay awake.

Several years after being first proposed (1), the concept of a tonic reticular control of wakefulness and sleep still maintains some validity. Any level of cerebral activity is probably related to, and actually maintained by, a given level of reticular activation. ARAS projects to the thalamus and excite cells, which in turn send fibres to widespread areas of the cerebral cortex to produce the cerebral

cortical activation that occurs during wakefulness. In addition, brain stem reticular neurons project to the hypothalamus and other regions of the basal forebrain. The neurons which project from there to the cerebral cortex also participate in the maintenance of an "alert" state of the brain. In addition, there are also fibres projecting to the cortex, directly from the brain stem reticular formation. Changes in single unit and integrated discharge of the reticular system undoubtedly occur during the sleep-waking cycle (5,6,7). Whenever an arousal phenomenon interrupts the state of SWS, or when a fit of rage occurs against a background of relaxed wakefulness, there is phasic increase in the ARAS barrage. The level of reticular activity is assumed to be higher during wakefulness than during sleep (8,9,10). Both the mild tonic activation required for the maintenance of wakefulness, and the strong phasic discharge responsible for rage outbursts, require the support of the ARAS. The sudden withdrawal of the activating reticular influence would produce a striking imbalance, which might be responsible for the coma of the acute cerveau isole. The statement does not imply that the ARAS exerts a nonspecific facilitatory control upon the cerebrum (11).

Sleep generating mechanisms in the brain stem

Structures functionally antagonistic to the ARAS have been discovered in the lower brain stem and in the cerebrum itself. At least two of these regions are tonically active, as shown by the fact that their inactivation produces striking hyposomnia. They are localized in the lower brain stem and in the forebrain area (preoptic area). Several experiments suggest that deactivation is an active process, probably related to inhibition of the ascending reticular barrage. There is some electrophysiological evidence to show that the hypnogenic structures inhibit the ARAS (12,13). There are interrelations between activating and deactivating structures, and they have functional significance in sleep inducing mechanisms. Sleep could be broadly divided into two categories, i.e. slow wave sleep and rapid eye movement sleep.

Slow wave sleep (SWS): Several lines of evidence indicate that the nucleus of the solitary tract (NTS) is involved in sleep generation. Distention of the carotid sinus, a powerful stimulus for the NTS region, induced behavioural sleep. Low frequency stimulation of the vago-aortic nerve (which carry impulses from aortic sinus) produced slow waves in the EEG. Electrical stimulation of the NTS produced synchronization of the EEG and behavioural sleep, though high frequency stimulation of the same region produced arousal (14). Inactivation of the lower brain stem regions, including the NTS, produces a profound arousal.

There is some evidence that certain NTS neurons increase their discharge during SWS and that these neurons are reciprocally connected with the cells in the midbrain EEG arousal region. Thus,

these data suggest that the NTS region may constitute a "center" for the regulation of SWS. However, there has been relatively little recent research in this area and the evidence for an NTS role in SWS control is weaker than that for the preoptic basal forebrain region.

Kumar et al (15) have localized the synchronizing structures at the nucleus gigantocellularis at the caudal brain stem. This study fulfilled the lacunae in our knowledge regarding the location of the regions in the caudal brain stem that bring about cortical EEG synchronization on electrical stimulation. It also characterized the features of synchronized waves elicited from those regions. Stimulation of ventromedial regions of the caudal brain stem, with low frequency, elicited stimulus-bound synchronized waves in the cortex, which were more prominent ipsilaterally. On the other hand, low-frequency stimulation of dorsal and lateral areas produced synchronized waves which were either equally prominent on both sides, or more prominent on the contralateral side. The induced synchronized waves showed amplitude modulation and did not outlast the train of stimuli (15). These evidences indicate the strong possibility that these caudal brain stem regions play an important role in the genesis of sleep.

Rapid Eye Movement (REM) Sleep: The primary role for the genesis of SWS is played by structures above the brain stem, but the activity of certain groups of neurons in the brain stem is important in the generation of REM sleep. Some of these neurons are characterized as REM-on neurons because of their selective activity during this state. Many of these neurons are located in the laterodorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei and use acetylcholine as a neurotransmitter. According to Datta (16), the cholinergic neurons involved in sleep-wakefulness are located at the peribrachial area (PBL). The major nuclei of the PBL are the PPT, the LTD, the cuneiform nuclei, and parts of the central tegmental field and paralemniscal tegmental field. The activity of PBL cholinergic and noncholinergic cells are also responsible for the EEG activation process during wakefulness and REM sleep. During wakefulness, together with noradrenergic and serotonergic cells of the brain stem, PBL cholinergic and noncholinergic cells activate the diencephalic EEG desynchronizing structures, which in turn activate cortical neurons. However, during REM sleep, only PBL cholinergic and noncholinergic cells are responsible for activating those diencephalic EEG desynchronizing structures.

Several findings suggest that there are two populations of cholinergic neurons which have different influences on sleep-wakefulness regulation. A population of cholinergic neurons in the brainstem which are most active during paradoxical sleep (PS-on neuron) are inhibited by carbachol and excited by bicuculline. Another population of neurons, which are active both during waking and paradoxical sleep, are inhibited by carbachol and excited by noradrenaline

and histamine. Non-cholinergic PS-on neurons are excited by carbachol and inhibited by noradrenaline (17).

According to McCarley (18) the LDT and PPT neurons activate the neurons located in the pontine reticular formation (PRF) which are the effector neurons for REM sleep phenomena. They begin to depolarize even before the onset of the polysomnographic signs of REM sleep. These neurons begin to discharge (i.e. generate action potentials) as REM sleep is approached, and the high level of discharge is maintained throughout REM sleep (18). PRF neurons are important for the rapid eye movements saccades and the PGO waves, which are the cardinal signs of REM sleep. A group of dorsolateral PRF neurons controls the muscle atonia of REM sleep, and these neurons become active just before the onset of muscle atonia. Neurons in the bulbar reticular formation are also important for muscle atonia.

Neurons in the locus ceruleus (using norepinephrine) and neurons in the dorsal raphe (using serotonin) have an opposite time course of activity compared to the LDT and the PPT neurons. They become selectively inactive during REM. They do suppress REM sleep-promoting activity of the LDT and the PPT neurons. These neurons, with an opposite discharge time course, are called REM-off neurons. REM-off neurons are most active in the waking state. Their discharge declines during slow-wave sleep, and are virtually silent during REM sleep. They resume discharge near the end of the REM sleep episode. This inverse pattern of activity to REM-off neurons has led to the hypothesis that these neurons may be REM-suppressive and interact with REM-on neurons in control of the REM sleep cycle. This concept is indirectly supported by production of REM sleep on cooling (i.e. inactivating) the nuclei where REM-off neurons are found. In vitro data from Luebke and co-workers (19) have provided direct support for the inhibition of cholinergic LDT neurons by serotonin.

Several classes of neurons are REM-off. Apart from norepinephrine-containing neurons in the locus ceruleus, and serotonin-containing neurons located in the raphe system, the midline collection of neurons that extends from the bulb to the midbrain, with serotonin-containing neurons in the more rostral regions, and histamine-containing neurons in the posterior hypothalamus, are REM-off. But transection studies indicate that the histaminergic neurons are not essential for REM sleep.

Role of thalamus in the genesis of sleep

There are three main systems in the brain which may be regarded as responsible for, or at least contributing to, the onset of sleep. They are localized respectively in the basal forebrain, in the lower brain stem, and in the midline nuclei of the thalamus.

According to Koella (20), the thalamamus is the head ganglion of sleep. Koella brings several interesting considerations in support of this hypothesis originally put forward by

Hess. Probably the most important among them is the striking similarity between natural sleep and the sleep induced by stimulation of the thalamus. Koella regards the hypnogenic regions of the basal forebrain, including the anterior hypothalamus, and of the lower brain stem, as subordinate sleep controlling structures. At the same time, he recalls that Hess (21) obtained a syndrome of generalized muscular relaxation, without typical sleep behaviour, from an area closely related to the basal forebrain. According to Steriade (22), brain stem, diencephalic and basal forebrain systems influence the functional modes of thalamic and cortical neurons during the behavioural states of vigilance. The posterior hypothalamus and the preoptico-anterior hypothalamus (PO-AH) can influence the neurons of the midline thalamus. A majority of the influenced neurons of the midline thalamus showed increased firing on stimulation of the posterior hypothalamus. Although the number of neurons showing increased or decreased firing on the preoptico-anterior hypothalamic stimulation were nearly equal, stimulus bound increased firing in many neurons was followed by a prolonged decreased firing. It is likely that the hypothalamo-thalamic circuit constitutes a parallel pathway to the reticulo-thalamic circuit for alteration of the cortical EEG (23).

Stimulation experiments of Hess and electrophysiological investigations on thalamic driving of electrocortical EEG spindles (24,25) had focused the attention of sleep physiologists on the thalamus for several years. Thalamocortical projection exhibits two distinct states of activity (a) synchronized rhythmic activity in the form of delta, spindle, and other slow waves during EEG-synchronized sleep and (b) tonic activity during waking and rapid-eye-movement sleep. Spindle waves are generated largely through a cyclical interaction between thalamocortical and thalamic reticular neurons involving both the intrinsic membrane properties of these cells and their anatomical interconnections (26).

The old literature on the effects of thalamic lesions was reviewed by Knott et al (27). A careful re-examination of these findings showed that the old experiments were contaminated by lesions of the hypothalamus and are therefore of little interest for the physiology of sleep. Naquet et al (28), who were able to follow six completely thalamectomised cats for up to 25 days, showed that the sleep-waking cycle was still present in these animals, although the EEG spindles were absent during behavioural sleep. Clearly sleep may occur even in the absence of the thalamus.

There is as yet no evidence of a marked hyposomnia produced by lesion of the midline thalamic nuclei(29). According to Moruzzi (29) the ARAS and a group of neurons lying in the posterior hypothalamus are probably concerned with the maintenance of wakefulness. The lower brain stem and the basal forebrain area contain structures with an opposing function, which exert a tonic deactivating influence and lead ultimately to sleep.

The role of hypothalamus in sleep-wakefulness

The ability to maintain a state of wakefulness do reappear in the chronic cerveau isole preparation. This shows that structures endowed with an activating influence are present in the cerebrum. They are mainly, though probably not exclusively, localized in the posterior hypothalamus. It is likely that these activating structures coincide with, or are at least strongly related to, the hypothalamic centre which is responsible for the outbursts of sham rage after decortication and for the defense-aggression behaviour of the normal animal.

Though it was known for years that there are centres or regions in the brain which could play an active role in the genesis of sleep, it was never given that much importance as to merit a place in the text books of neuroscience and physiology. In fact it was the statement of Moruzzi himself, in his review article which appeared in 1972, which played a major role in informing the world about the importance of the preoptic area (POA) in the genesis of sleep (29). Attempts have been made to locate within the POA the critical area for genesis of sleep. But the areas involved are not likely to restricted within this area. So, people have referred to this hypnogenic area as the basal forebrain or the anterior hypothalamus. But some others have referred to the area as the preoptic-anterior hypothalamus (PO-AH). In this manuscript, an attempt is made to refer to the areas using the terms mentioned by the authors themselves. Information gathered about the role of the hypothalamus in sleep-wakefulness is listed under different techniques employed in collecting the evidences.

Brain lesions and section studies: von Economo (30) was the first to draw the attention of physiologists to the involvement of the rostral hypothalamus in sleep, on the basis of his observations in some cases of encephalitis. On post-mortem examination of brain material collected from cases of *encephalitis lethargica*, he described two symptomatic patterns of the disease, associated with two different localizations of inflammatory lesions in the nervous system. In those cases in which somnolence was the distinguishing symptom, the lesions were regularly found in the posterior wall of the third ventricle, continuing caudally to the level of the occulomotor nucleus. In contrast to this, there were other cases in which insomnia was observed. Inflammation in these latter patients was associated with the rostral hypothalamus, the tuberal region and the adjacent portion of the striatum. From these observations, von Economo concluded that the rostral hypothalamic zone was a part of a "sleep regulating center" which, when appropriately excited, actively inhibited the thalamus and cerebral cortex and caused "brain sleep". Therefore, he concluded that the rostral hypothalamus is a "sleep center". In 1936, Ingram and co-workers (31) reported that in cats a lesion between mammillary bodies and the third nerves, which involved the caudal hypothalamus and the upper part

of the mesencephalic tegmentum, led to a state that resembled catalepsy. In 1939, Ranson (30) demonstrated in rhesus monkeys, that bilateral lesions in the lateral hypothalamus which extended up to the mammillary bodies, produced a lethargic syndrome. He reported that there was a lack of motor initiative and the symptoms were similar to those observed in catalepsy.

After von Economo's observations (30), several studies were carried out to elucidate the role played by the POA in the regulation of sleep. In 1946, Nauta (33), employing the knife cut lesion technique, showed that the rats became insomniac, restless and irritable after the lesion of the POA. They reacted vigorously even to minor stimuli. Those rats which survived up to 13 days did not show a return to what he called the "capacity of sleeping". He thought that the POA was an important region for sleeping and he termed this area as "sleep centre". Nagel and Satinoff (34) reported hyperactivity in rats after bilateral electrolytic lesion of the mPOA. McGinty and Sterman (35) reported that large bilateral POA lesions produced complete sleeplessness in cats. Smaller lesions resulted in significant reduction in SWS as well as in REM sleep. The severity of sleep suppression was found to be related to the size and localization of the lesions placed specifically within the POA (36). These lesions shortened the mean periodicity of the sleep awake cycle with a decrease in SWS and no alteration in REM sleep (36). Neurotoxic lesion studies provided convincing evidence of the involvement of the POA neurons in the regulation of sleep. Lesions produced by neurotoxins such as kainic acid, which spares the fibres of passage in the medial and lateral POA, reduced both SWS and REM sleep (37,38). Suppression of sleep produced by the NMDA lesion of the medial preoptic area (mPOA) neurons showed the importance of this area in the regulation of sleep (39). Persistence of hyposomnia for three weeks after the lesion further showed that the deficits produced by the mPOA lesions were not compensated for. Insomnia was primarily due to a reduction in the duration of SWS episodes. There was a trend towards a reduction in the frequency and duration of REM sleep episodes. Recovery of sleep in the lesioned rats, after the fetal neural tissue transplantation, indicated the vital role of the mPOA in the regulation of sleep (40).

c-Fos expression studies: c-fos expression is strongly induced by both spontaneous and forced wakefulness in many brain regions. c-Fos expression was considerably increased in regions involved in the regulation of S-W, such as the locus coeruleus and the mPOA. With c-fos antisense injection in the mPOA, it was demonstrated that c-fos expression in this region is causally involved in sleep regulation. c-Fos expression in other areas, such as the cerebral cortex and the hippocampus, are explained as related to the functional consequences of prolonged wakefulness and to the need for sleep (41). Periods of wakefulness result in the induction of immediate-early gene c-fos in the mPOA. Injections in the rat mPOA

of c-fos antisense, oligonucleotides, blocked the expression of Fos protein detected immunocytochemically. Rats receiving bilateral antisense injections showed a higher percentage of wakefulness, the day after the injection, than controls receiving sense or sham injections or antisense injections outside the POA. These results suggest that blocking the expression of fos protein in the POA may interfere with the homeostatic regulation of sleep and wakefulness (42). Immunocytochemistry was used to identify the fos protein, an immediate-early gene product, in a group of ventrolateral POA neurons that is specifically activated during sleep. The retrograde tracer cholera toxin B, in combination with fos immunocytochemistry, was used to show that sleep-activated ventrolateral POA neurons innervate the tuberomammillary nucleus, a posterior hypothalamic cell group, thought to participate in the modulation of arousal. This monosynaptic pathway in the hypothalamus may play a key role in determining sleep-wake states (43).

Stimulation studies: Sterman and Clemente (44), on the basis of behavioural and electrophysiological observations, reported that bilateral stimulation of the POA in unanaesthetised freely moving cats, produced sleep. Low frequency stimulation was effective in inducing sleep. The effect of low frequency stimulation (5-25 cycles/sec) on induction of sleep was confirmed by Hernandez-Peon (45), and Yamaguchi et al (46). In addition, they showed that high frequency stimulation (200-300 cycles/sec) induced cortical EEG desynchronization and some signs indicative of behavioural arousal. Thus, it can be concluded that both sleep and arousal responses can be obtained from electrical stimulation of the POA, depending upon the rate and site of stimulation.

Local intracerebral injection studies: Injection of norepinephrine (NE) at the mPOA produced arousal and the alpha receptors were involved in this response (47,48). Locally applied NE can act on both the presynaptic and postsynaptic receptors (49). It was later shown that the administration of NE did not induce arousal when the noradrenergic fibres in the mPOA were lesioned (49). These findings showed that the NE induces arousal by acting on the presynaptic alpha-2 receptors. Stimulation of alpha-2 receptors by externally applied NE and clonidine, would produce decreased release of endogenous NE from the presynaptic terminals (50). So, it is very likely that the decreased release of endogenous NE produced arousal in normal animals. This assumption was further supported by the finding that the alpha-2 agonist, clonidine, also produced arousal when applied at the mPOA (51). Alpha-2 receptors are primarily present on the presynaptic noradrenergic terminals (50,52). The presynaptic site of the action of clonidine was confirmed by the studies on VNA lesioned animals (53). Similarly, yohimbine, an alpha-2 antagonist, induced sleep by its action on the presynaptic terminals, as the effect was attenuated after the VNA lesion. Thus, the observations in the VNA

lesioned rats further confirmed the contention that these responses are mediated through presynaptic alpha-2 terminals (49,51).

Injection of beta adrenergic blocker, propranolol, at the mPOA was ineffective in changing sleep-wakefulness (47,54). But, beta receptors are also thought to be indirectly involved in the regulation of sleep-wakeful function. Beta agonist, isoproterenol injection into the mPOA induced wakefulness (55). Beta receptors in the mPOA have been shown to be important in inducing sexual arousal (56), which would in turn cause generalized arousal. So, it is possible that the injected isoproterenol would have produced arousal through a stimulatory action on the sexual arousal system. It may be hypothesized that there are different sets of neurons in the mPOA, controlling sleep, sex drive and other functions. It could be possible that the sleep regulating neurons are primarily stimulated through alpha receptors, whereas the neurons controlling the sex drive are activated through beta receptors.

Progesterone has also been reported to induce sleep, when applied directly into the rostral POA or basal forebrain area in cats, whereas no change was observed when it was injected into the caudal or lateral regions (57).

Prostaglandin has been shown to alter sleep by its action at the POA Prostaglandin D2 (PGD2) is present in high concentration in the hypothalamus in the rat brain. Ueno et al (58) reported that PGD2 application into the POA of conscious rats increased SWS. On the other hand prostaglandin E2 (PGE2) produced arousal in rats (59,60). According to Hayaishi (61) PGD2 is the endogenous sleep inducing substance in rats, mice, monkeys and probably in humans. PGD synthase (PGDS), the enzyme that produces PGD2 in the brain, is the key enzyme in sleep regulation. When the enzyme activity is inhibited by its specific inhibitor, SeCl4 in vivo, rats can no longer sleep. PGDS is present mainly in the arachnoid membrane and choroid plexus. It is secreted into the cerebrospinal fluid to become beta-trace. PGD2 thus produced is bound to the receptors on the surface of the ventro-medial region of the rostral basal forebrain. This signal is probably transmitted into the brain parenchyma by adenosine via adenosine A2a receptors. PGE2 plays a major role in the maintenance of wakefulness.

Hernandez-Peon (62) showed that the application of ACh at the POA elicited EEG synchronization and sleep, in cats, whereas carbachol, a cholinergic agonist, when applied at the mPOA, produced a fall in rectal temperature and an injection bound long lasting arousal in rats (63). Administration of GABA at the mPOA did not produce any significant alteration in S-W (64). Application of 5-HT crystals in the POA produced drowsiness and SWS in freely moving rats (46). But, Datta et al (65) showed that 5-HT application at the same site did not have any change in SW. Intracerebral injection of melatonin into the POA has been shown to increase SWS and REM sleep in rats (66).

Single unit recording studies: Mallick et al (67) showed that a majority (55%) of neurons of the POA showed alterations in their firing rate during transient changes in EEG. Among these 62.5% showed increased firing during synchronization and the remaining 37.5% showed increased firing during desynchronization of the EEG. Findlay and Hayward (68) showed that the majority of the neurons in the hypothalamus, including POA in freely moving rabbits, showed an increase in their firing rates during sleep as compared to the awake state. The POA neuronal activity was recorded during all states of sleep and wakefulness, and were classified into five groups according to their firing behaviour in relation to sleep-wakefulness states (69). One third of the neurons showed no clear correlation with the sleep-waking states. Out of the 65 neurons which showed changes in activity with sleep-wakefulness states, 26 were most active during REM sleep. There were 16 neurons which were most inactive during REM sleep, some of which were inactive during SWS also. Those which were specifically active during SWS were 14 in number. Nine were less active during SWS than during wakefulness and REM sleep. About one third of neurons which showed increased discharge with SWS and REM sleep, began to increase their sleep-related activity in advance of the shift of sleep-wakefulness state recognized in EEG. These results suggest that the PO-AH areas are involved, at least in rats, in regulation of not only SWS but also REM sleep.

The role of the mPOA in the maintenance of sleep-wakefulness

There was persistent reduction in sleep (light SWS, deep SWS and REM sleep) and increase in the awake periods in rats after the mPOA neuronal lesion using NMDA (38). In cats, light SWS was not much affected after electrolytic and neurotoxic lesion (35,70). So, there was reduction in deep SWS and REM sleep in both rats and cats, after the mPOA lesion (39,70). The reduction in REM sleep suggests that the integrity of the POA is important for the regulation of this phase of sleep also (70,71). It is worth noting that the majority of the POA neurons show higher firing rates during REM sleep, than during SWS (69,72).

There was a significant decrease in the duration of SWS episodes after the mPOA lesion in rats (39). There was no significant increase in SWS frequency after the mPOA lesion. Thus, the initiation of sleep was not affected significantly by the mPOA lesion. This indicates that it is the maintenance of SWS rather than its initiation, which seems to have been mainly affected after the mPOA lesion. Lucas and Sterman(36) have also reported that there was a decrease in SWS bout duration, and an increase in the number of transitions of sleep stages after the basal forebrain lesions in cats.

There was a reduction in sleep pressure after the mPOA lesion, which shortened the sleep episode duration (39). This produced a reduction in the duration and frequency of deep SWS and REM

sleep episodes. Normally, deep SWS occurred after the animal had spent some time in slow SWS. Similarly REM sleep appeared after the animals had spent some time in deep SWS. Thus, the reduction in sleep episode duration produced a reduction in frequency and duration of deeper stages of sleep, namely deep SWS and REM sleep. Unlike in human beings, in rats and cats, REM sleep forms a part of the deeper stage of sleep. So, the studies on NMDA lesioned rats indicate the possibility that the mPOA neurons are important for the maintenance and initiation of REM sleep.

Marginal increase in the frequency and duration of awake episodes, after the mPOA lesion (39), could be due to a release of the waking mechanism from the inhibitory influence of the POA (73).

Role of the mPOA in circadian and sleep-wake rhythms

Circadian rhythms are major features of the adaptation to our environment (74). In mammals, circadian rhythms are generated and regulated by a circadian timing system. This system consists of entertainment pathway, pace-maker, and pace-maker output to effector systems that are under circadian control. The primary entertainment pathway is the retinohypothalamic tract, which terminates on the circadian pace-maker, the suprachiasmatic nuclei of the hypothalamus. The output of the suprachiasmatic nuclei is principally to the other nuclei of the hypothalamus, the midline thalamus, and the basal forebrain. This provides a temporal organization to the sleep-wake cycle, in addition to many other physiological, endocrine and psychomotor functions (74).

In young adult human beings, the sleep-wake cycle coincides with the circadian cycle. But in cats and rats, and even in children, they do not exactly coincide. They are polycyclic and go through several cycles of sleep during the day and night. But all the same, they do show a circadian variation in the amount of sleep during day and night. The effect of brain lesion on sleep-wakeful cycle could be better studied in polycyclic animals, where the circadian cycle and the sleep-wakeful cycle could be viewed separately.

Many regions of the brain have the ability to produce arousal, or sleep. But it is necessary to locate the brain areas essential to generate the normal pattern of sleep-wake cycle. Neural regulation of sleep is highly incomplete without a proper regulation of sleep-wake cycle itself. Study of the sleep-wakeful cycle would also help in looking at the change in the duration and frequency of sleep episodes, as mentioned in earlier section. The sleep-wake cycle could be due to the slow accumulation and dissipation of chemical products within well defined groups of neurons in the brain (75). Rhythmicity is potentially present both in the cerebrum and in the brain stem, as shown by the experiments on the chronic cerveau isole and chronic decerebrate preparations (29). It is difficult to state where rhythmicity arises in the normal

animal, but several considerations suggest that the cerebrum, especially the hypothalamus, is the most likely location. According to one theory the sleep-waking cycle would normally arise within the cerebrum, and they are controlled by the ascending flow of brain stem impulses from the ARAS and the sleep inducing structures of the lower brain stem. But the important role of the hypothalamus for sleep-wakefulness change is particularly emphasized by Kawamura (76) on the basis of experimental data. According to him the mechanism of sleep-wakefulness change, produced by the forebrain, does not depend on the lower brain stem structures and ARAS. One week after rostral midbrain transection, the isolated forebrain showed sleep-wakefulness change, with circadian rhythm. In this preparation, after additional bilateral preoptic or posterior hypothalamic lesions, ECoG "insomnia" or "coma" pattern appeared, respectively. Hypothalamic sleep-wakefulness mech-anism usually receives strong influence from the suprachiasmatic nucleus, but it can produce its own ultradian "rhythms" (sleep-wakefulness change), though very irregular, even without this input.

The mPOA lesion in rats produced a proportionate reduction in sleep during the day and night, without any obvious change in the day-night sleep ratio (39). Absence of any persistent change in the night-day ratio of sleep would suggest that the mPOA has no role to play in the circadian distribution of sleep. Asala et al (77) have reported an uneven suppression of sleep after the radiofrequency lesion of the mPOA. In those rats the reduction in the light period sleep was compensated for by the dark period sleep. It is difficult to assign any reason for the differences in the observations in the radiofrequency lesioned and the neurotoxic lesioned rats. The damage to the input from suprachiasmatic nucleus in the radiofrequency lesioned rats, could have been instrumental in disrupting the night-day distribution of sleep. Night-day distribution of food and water intake was also not significantly altered after the mPOA lesion. Though there was no alteration in night-day sleep-wakefulness, food and water intake, the rest-activity cycle was disturbed by the mPOA lesion.

Inter-relationship between hypothalamic thermoregulatory and sleep regulatory mechanisms

Roberts and Robinson (78) have suggested that the POA thermoreceptors may provide input to the POA sleep-regulating mechanisms. Stimulation of central receptors by changing blood temperature is likely to be an important source of impulses driving the sleep inducing structures of basal forebrain (30). It was hypothesised that the SWS in mammals and birds is controlled by thermoregulatory mechanisms (79). Local warming of the POA produces sleep (78,79,80). PO-AH warming increases EEG delta frequency activity during SWS (80). So, it was suggested that the PO-AH thermoregulatory mechanisms participate in the regulation of the depth of SWS. According to Nakao et al (81) the SWS is controlled by thermoregulatory mech-

anisms of the PO-AH. Circadian and homeostatic thermoregulatory processes may be integrated in this brain area. Sleep could be induced by radio frequency diathermic warming of the POA in cats and opossum (78). Cooling the POA produces huddled posture (82). Low ambient temperature suppressed sleep and mild environmental warming enhanced sleep in normal rats (83,84). Studies have shown that SWS is facilitated when brain temperature exceeds a threshold level (79). This threshold is hypothesized to be determined by responses of the PO-AH thermosensitive neurons and to be regulated by both circadian and homeostatic processes. SWS-induced brain and body cooling would provide several adaptations including lower energy utilization, reduced cerebral metabolism, protection of the brain against the sustained high temperatures of wakefulness, facilitation of immune defense processes and regulation of the timing of behavioural activity relative to the circadian light-dark cycle. So, it was suggested that the mPOA, anterior hypothalamic and basal forebrain network integrates thermor-egulatory and hypnogenic controls and induces EEG and behavioral deactivation, through suppression of the ARAS.

It has been demonstrated that there are neurons in the mPOA involved in the regulation of sleep and body temperature (85,86,87). Thermosensitive neurons of the PO-AH have been implicated in the regulation of both body temperature and SWS (88). The activation of sleep-related warm-sensitive neurons (WSN) and the deactivation of wake-related cold-sensitive neurons (CSN) may play a key role in the onset and regulation of SWS (89). During SWS, a majority of WSN of the PO-AH exhibit increased discharge compared to wakefulness. CSN exhibit reduced discharge in SWS, compared to wakefulness. WSN with increased discharge in SWS exhibited increased thermosensitivity during SWS compared to wakefulness. CSN with decreased discharge during SWS exhibited decreased thermosensitivity in SWS. In addition, 9 out of 47 neurons that were thermo-insensitive during wakefulness became warm-sensitive during SWS. Changes in PO-AH neuronal thermosensitivity could be a component of the mechanism for stabilization of state after state transition (88).

WSN did not exhibit a significant change in thermosensitivity during REM sleep compared with wakefulness and SWS (90). In contrast, CSN exhibited decreased mean thermosensitivity during REM sleep compared with wakefulness. CSN as a group did not retain significant thermosensitivity in REM sleep. These findings are consistent with evidence that thermoeffector responses to cooling are lost in REM sleep, whereas some responses to warming are preserved (90).

The mPOA lesions do increase the rectal temperature (70,71,91,92). Hyperthermia observed during the first week after the mPOA lesion was severe. This was followed by a constant mild hyperthermia during the subsequent weeks (39,92,93). On the other hand, there was

no variation in the magnitude of reduction in sleep throughout the post-lesion period. Thus, there was no temporal correlation between the sleep and temperature changes after the mPOA lesion. Though hyperthermia is the commonly reported observation, hypothermia was also reported after the lesion of the POA (94,95).

Though the possible strong interrelationship between the regulations of body temperature and sleep-wakefulness has been suggested on the basis of single unit and local warming studies (79), they are not supported by intracerebral injection studies. Neurotransmitters and their antagonists, injected at the mPOA, could not always produce simultaneous alterations in sleep and body temperature (48,63,96). Carbachol administration at the mPOA produced hypothermia and arousal. But the changes in these physiological parameters did not have a temporal correlation. The arousal response outlasted far beyond the changes in body temperature (63). Administration of 5-HT at the mPOA produced hyperthermia without any change in sleep-wakefulness (65). So, it is possible that the changes in sleep-wakefulness resulting from the mPOA stimulation and lesion are not dependent on the body temperature changes. Thus, it may be suggested that the mPOA controls sleep and temperature through independent, but overlapping, neuronal circuits. This conclusion is also supported by the observations of Krueger and Takahashi (97).

Effects of noradrenaline (NA) on the activity of sleep-related neurons in the POA and the neighboring basal forebrain were examined in the rat by Osaka and Matsumura (86). Sleep-active neurons were generally inhibited by NA and the alpha 2-agonist clonidine, whereas the alpha 1-agonist methoxamine and the beta-agonist isoproterenol had no effect on them. Thus, alpha 2-receptors mediated the NA-induced inhibition. Waking-active neurons were excited by NA and methoxamine, whereas isopro-terenol and clonidine were without effect. Accordingly, alpha 1-receptors probably mediated the NA-induced excitation. State-indifferent neurons, and REM sleep-active neurons were mostly insensitive to NA. These results suggest that NA promotes wakefulness by inhibiting sleep-active neurons and by exciting waking-active neurons (86).

The noradrenergic terminals, when activated, bring about sleep and hypothermia. On the basis of local application of NA, it has been postulated that there are also two separate groups of afferent noradrenergic inputs, ending on the mPOA neurons. One of them, terminating on sleep inducing neurons, is tonically active during sleep. Those afferents which synapses on the temperature regulatory neurons are suggested to be normally inactive (53). Clonidine administration at the mPOA produced arousal (51), but it was ineffective in producing any change in temperature (98). Clonidine (alpha-2 agonist) injection into the mPOA, in

normal rats, resulted in the activation of presynaptic alpha-2 receptors, on both the groups of noradrenergic afferents, but it brought about decreased release of endogenous NA on those neurons in which there was a tonic release. This decreased release of endogenous NA produced arousal in sleeping animals (51). Clonidine also acted on the inactive terminals which synapse on the temperature regulatory neurons. Since these fibres normally secrete very little NA, there was no change in the body temperature when this drug was applied. Yohimbine, an alpha-2 antagonist, blocks the presynaptic receptors and facilitates the release of endogenous NA. Postsynaptic action of the released NE on alpha-1 receptors, induces sleep in normal animals (51,99,100). Yohimbine failed to exert facilitated release of NA from those fibres which synapse on to the temperature regulatory neurons, since they are normally inactive. Hence, there was no change in the body temperature on application of this drug.

Changes in body weight, food and water intake on sleep regulation

Earlier reports have shown that the alteration in food intake can disrupt sleep (101). There are reports in the literature which indicate that the REM sleep deprivation or total sleep deprivation increases the food intake (102,103,104). But the decrease in SWS and PS, resulting from the mPOA lesion, did not produce any increase in food intake and water intake (39). Food deprivation in birds and squirrels resulted in a lowering of the thermoregulatory set point during sleep along with increased SWS (105).

Though there was no significant persistent change in food intake, there was a reduction in the body weight of the rats after the mPOA lesion with NMDA, and electrolytic lesion of the POA (39,84). Higher locomotor activity and increased body temperature, after the mPOA lesion, would produce increased energy expenditure. This might have resulted in a decrease in the body weight because there was no concomitant compensatory addition in energy intake (food intake), in spite of the increase in locomotor activity, rectal temperature and awake period. Therefore, after the lesion, the animal did not recognize low energy reserves, and so it did not bother to conserve energy. Thus, it can be hypothesized that the mPOA lesioned animals had lost the mechanism for the fine tuning of food intake regulation in response to the alteration in body homeostasis. The functional integrity of the mPOA may be essential for the regulation of food intake, in response to alterations in the temperature, locomotor activity and S-W. It can also be argued that the POA would normally facilitate sleep, an energy-conserving state, when energy reserves are at a critical level.

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Neural Transplantation

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INTRODUCTION

In recent years nothing in the field of surgery has captured the imagination of common man more than the subject of organ transplant. Ever since Christian Bernard successfully transplanted a human heart, not only the lay public but even the members of the scientific community have often asked us. "When will a brain transplant be done?" A decade or so ago, the simple answer would be "Never". However, much has happened during the past decade to make us a bit more circumspect in giving a categorical answer. Thousands of neuroscientists all around the world, including a group in our own Institute, have been currently working hard to explore various possibilities leading to such a goal. A large number of publications both in scientific and lay press refer to the tantalising possibilities of at least replacing part of the damaged or diseased brain, by viable neural tissue, with capabilities to grow, differentiate, develop connections with the host brain, produce chemicals responsible for transmission of nerve impulses and ultimately compensate for the lost function. It is therefore timely to take stock of the current status and future perspectives.

Why the Whole Brain cannot be Transplanted

It is natural to question that if heart, liver, lungs, kidneys can be transplanted as an 'organ' why brain cannot be transplanted in the same manner. Heart, liver and kidney are connected with the body primarily through blood vessels, which can be divided and sutured to the vessels of the donor organ. Similarly the ureter or bile duct which have to be divided while removing a kidney or liver, can be easily stitched to the appropriate tissue of the donor. These structures i.e., blood vessels, ureter or bile duct are simply mechanical conduits which can be sutured easily and successfully. In contrast, in addition to the blood vessels, the brain is connected to the body by the spinal cord. The donor brain will thus be required to be anastomosed with this

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structure, which is not easy to be sutured. Even if it is sutured, it has no capacity to regenerate, or, to develop functional connection with the donor brain. In addition, the brain is connected to the body by twelve pairs of cranial nerves which would have to be cut if brain is to be removed. Some of these, like the optic nerves connecting the eyes to the brain, even if connected to the nerve of the donor brain, somehow fail to become functional.

The next best thing to full brain replacement is therefore replacement, if possible, of a part of the brain which is diseased or damaged. This is what is currently being tried.

Historical Background

Historically, attempts at mammalian neural transplantation started nearly one hundred years ago. Thompson in 1890 attempted exchange of large pieces of brain tissue between adult cats and dogs. Saltykow in 1905 reported the survival of transplanted mammalian cortical tissue. Nevertheless, it soon became obvious that adult neural tissue seldom survived and that too for a very short duration. Dunn in 1971 demonstrated that immature neural tissue had longer survival. However, it was only in 1940 that Le Gros Clark successfully transplanted embryonic brain tissue in the cerebral ventricle of a six week old rabbit. Four weeks after transplantation, he demonstrated well differentiated neurons attempting to reproduce the laminar arrangements of adult cortical neurons. Sporadic efforts by a series of workers (May 1954, Greene and Arnold 1945 : Royo and Quay 1959) reconfirmed the capability of embryonic neural tissue to grow when transplanted in anterior chamber of the eye in adult host. On the other hand, Willis (1935) and Glees (1955) could not confirm the ability of embryonic neural transplants to survive. However, concerted efforts in this direction started only in early seventies, when the work of Das and Altman from USA and Bjorklund and his colleagues from Sweden in a series of publications unequivocally demonstrated growth, differentiation, integration and even production of appropriate neurotransmitters following embryonic neural tissue transplanted in various regions of the brain in adult rats (Das 1983). These successes have resulted in a virtual explosion of researches in this field so that thousands of neuroscientists all over the world are currently engaged in this field of study. According to Morrison (1987), in 1983 alone, as many papers were published on neuronal grafting in mammals as in the whole century preceding 1970.

Need for Neural Transplantation

Brain cells - the neurons - have no capacity to regenerate when they are damaged. In addition, groups of neurons and their interconnections constitute functional systems with specific functions. Damage to any component of a particular system leads to loss of that function. Brain has limited capacity to delegate or transfer this function to any other region. Just to give an example, if the area of the brain concerned with speech is damaged, the patient loses his speech. The remaining

healthy brain cannot take over this function. This is true for other specialized functions like seeing, hearing, voluntary movements, sensory perception etc. The individual units of a functional system communicate with each other through electrical signals which utilise specific chemicals called neurotransmitters. Some neurotransmitters are also specific for a particular functional system. Thus for maintenance of smooth motor functions, besides the motor cortex and cerebellum, the nigrostriatal system is essential. The specific neurotransmitter that subserves this system is dopamine. Deficiency of dopamine results in abnormal motor symptoms like poverty of movement (akinesia), stiffness or rigidity and involuntary movements (tremors) so characteristic of Parkinson's disease. No other neurotransmitter, of which there are a large number, can take over the function of dopamine. In contrast, in the case of liver or kidney, all the liver cells (hepatocytes) or kidney cells (nephrons) have identical functions. When diseased, remaining healthy cells can take over the function. Furthermore, upto a certain limit these organs have an inherent capacity to regenerate. Brain unfortuately does not possess any of these characters. Hence, once any specific part of it is damaged, it can only be replaced by those cells which have genetically been ascribed that particular function or which are able to produce the specific neurotransmitter involved in that function. To make matters more complicated, for some complex functions, large number of functional units are required to work in harmony and this may involve more than one transmitter. This is characteristically seen in respect to memory. Impairment of memory, so characteristic a feature of ageing, especially in cases of Alzheimer's disease, represents such a situation.

Theoretically speaking, sequelae of injury, infections, stroke and a large number of degenerative disorders of brain which have no medical or surgical therapy available for restoration of function, are all potential candidates for neural transplantation. Experimental neural transplantation in animal models of a host of such disorders has been shown to benefit to a varying degree the impaired function. Thus, neural tissues from a number of morphologically, biochemically and functionally identified regions have been "harvested" from foetal brain and successfully transplanted in the appropriate region of the adult brain with pre-existing mechanical, biochemical or pathological lesion. These grafts have been shown to develop organotypic maturation, produce appropriate neurotransmitters and result in varying degree of recovery of lost function. However the procedure has so far been utilized for treatment of Parkinson's disease only. The remaining portion of this article will predominantly deal with this disorder.

It is now well established that the dominant abnormality in patients with Parkinson's disease involves the nigrostriatal dopaminergic neurons. A variety of experimental models of this disorder have been developed in animals by destroying the dopaminergic neurons either chemically or mechanically. It is

possible to isolate dopaminergic neurons from either embryonic brain or adult adrenal medulla. These neurons have been transplanted in the lesioned animals. Morphological, Neurophysiological, biochemical, histochemical and behavioural studies have unequivocally demonstrated that such transplants are able to revert the lesioned animals towards normalcy (Bjorklund et al 1981, Schmidt et al 1982, Dunnett et al 1983). This knowledge has already been utilized to treat patients suffering from Parkinson's disease (see later).

Two decades of intensive research all over the world has established that the most suitable tissue for grafting in the brain and spinal cord is the fresh neural tissue obtained from a developing foetus. Atleast in lower mammals one can easily achieve a success rate of 80 to 85 percent. Most such studies have been carried out in rodents. These findings have also been confirmed in a host of other animal species including sub-human primates and even man. However, the work carried out in monkeys has been very limited (Ridley and Baker 1991) and the work carried out in rhesus monkey has been far from satisfactory (1992). It is therefore not surprising that several investigators have questioned the advisability of using this procedure in man on the basis of results obtained in rats (Fishman 1986, Tandon 1988, Sladek and Gash 1988, Sladek and Shoulson 1988, NMJ 1988, Lindvall 1991). Inspite of such reservations, it is surprising that more than 400 human patients have been subjected to such transplants, using either autologous adrenal medulla or foetal substania nigra. Such "experimental" surgery has no doubt demonstrated the feasibility of the procedure to succeed and atleast partially and temporarily alleviating some of the deficits resulting from Parkinson's disease. The developmental window during which the fetal cells need to be harvested is reasonably well established for rodents. The optimal donor age may vary depending upon the region of the brain selected for transplantation. On the other hand, while most persons believe that in the case of human foetuses, the desirable age to take the graft is from 6 to 12 weeks.

Successful transplantation has been achieved using cell suspension or solid pieces of foetal tisue. Bjorklund et al (1980), Dunnett et al (1987) and many others advocate cell suspension to be better than solid pieces. Das et al (1979) and others including us, find the latter to grow as well. The size of the graft in animal experiments was not a critical parameter, but it acquires great importance when the technique is used for therapeutic purposes in human patients. Lindvall et al (1987) estimated that the human putamen and caudate nucleus (striatum) are normally innervated by about 60,000 dopaminergic neurons each. Grafting ventral mesencephalic tissue from one fetus into one of these structures might then be able to restore upto 30%-40% of the normal number of cells. Hence, they used tissue from four foetuses for each of their patients (Lindvall et al 1990), though Madrazo et al (1991) and Hitchcock (1991) claimed satisfactory results using cells from a single fetus.

It is now obvious that, for proper functional integration, the graft must be located as near the target as possible. However, the problem arises when one deals with such a large structure as striatum. With the restricted axonal growth into the host and limited diffusion of dopamine into the surrounding brain, it appears to be desirable to transplant at several sites and not at one (Perlow 1987). Madrazo et al 1990). Furthermore. It has been demonstrated that selected aspects of behavioural abnormalities are benefited by transplants at different sites in the caudate nucleus (Dunnett et al 1989, Perlow 1987). Efforts are, therefore, underway to evaluate the utility of transplants in only caudate nucleus or putamen or both unilaterally or bilaterally, at single or multiple sites in cases of Parkinson's disease (Freed et al 1990, Hitchcock et al 1989).

It must be stated at the outset that neural transplant has not been attempted in India so far inspite of the fact that we have extensive experience in achieving successful transplantation of foetal tissue in adult animals. The technique itself is simple, does not require any sophisticated equipment and can be performed with little, if any risk to life. There will be no dearth of patients, the cost of surgery itself would be affordable by a common man. The reasons for not attempting it is, however, based on its purely experimental nature as at present. In addition results of our animal studies as also a host of others entreat us to resolve some of the controversial and unanswered questions through experimentation in animals. Some of these are briefly described below.

Donor Tissue

As mentioned above, so far, the only suitable tissue necessary for successful transplantation is human foetal tissue, that too from a foetus of a particular age. To procure such tissue raises some practical and ethical issues notwithstanding the fact that abortion having been legalized in the country, thousands of such procedures carried out every day could easily provide the required donor tissue. The current practices of medical termination of pregnancy may need to be modified to procure the desired donor tissue in suitable condition. The donor tissue thus obtained will have to be transplanted promptly.

Preservation of Graft

Most investigators studying neural transplant used fresh donor tissue. For clinical use, specially keeping in mind the need for tissue from more than one foetus for a patient, it would be more practical to use stored tissue. Attempts have been made to use cryopreserved tissue (Brundin et al 1985, Redmond et al 1988, Gibbs et al 1986, Victorov and Lyjin 1990). We were able to successfully transplant cryopreserved foetal tissue in rhesus monkey (Tandon et al 1990). However, Gash and Sladek (1989) observed that survival of human foetal cells whether cryopreserved or dissociated in-vitro is still extremely low (5% to 15%). A proper protocol needs to be developed to achieve better survival. In addition one would have to ensure that tissue thus obtained is free from any infection, specially the human immunodeficiency

virus [HIV], and is not contaminated during collection, transportation and preservation. In view of these problems, efforts are being made to look for satisfactory alternatives (vide infra). However, before we do that we may have a look at what has already been achieved using human foetal tissue transplantation for Parkinson's disease, since this is the only disease for which this procedure has been utilized and very carefully studied.

The first operation, using autologous adrenal medullary cells was performed by Backlund and his colleagues in March 1982. A second operation was performed in May 1983. A suspension of chromaffin cells of the patient's own adrenal medulla which secrete a precursor of dopamine was stereotactically implanted into the caudate nucleus. The transitory and equivocal improvement observed in these patients, while establishing the validity of such an approach, dictated a review of the strategy (Backlund et al 1987). Madrazo and his colleagues in Mexico, modified this operation and claimed dramatic results. They carried out an open operation, exposed the caudate nucleus in the lateral ventricle, made a cavity in it and implanted adrenal medulla into the cavity. Few others could verify their dramatic results and soon the operation fell into disrepute. This promoted Hitchcock et al (1989), Lindvall et al (1989), Madrazo et al (1990) to use foetal substantia nigra neurons (ventral mesencephalon of embryo of 8 to 10 weeks gestation) for transplantation. Till date, this is the only transplant which has provided symptomatic relief lasting atleast for more than 2 years in selected patients.

The beneficial effects of nigral transplants may be summarised as follows :

- Some general improvement of motor symptoms has been reported by all. However, the degree of improvement is rated as mild to moderate. In no case there has been a full reversal of Parkinsonian syndrome (Lindvall et al 1990, 1992).
- The most consistent improvement has been in rigidity and bradykinesia. There is little improvement in tremors.
- Positron emission tomography [PET] scan studies have established long term survival of the graft (Lindvall et al 1990, 1992).
- Restoration of dopamine synthesis and storage in the grafted striatum has been established using an isotope, fluorodopa [6 FD PET] uptake (Sawle et al 1992). Vingerhoets et al (1994) summarising the current information based on 6 FD PET studies concluded that some of the relevant reports currently available are highly encouraging but *"there are also inconsistent, paradoxical and controversial findings"*.

Future Directions

To overcome the drawbacks and limitations of foetal tissue transplant, efforts are being made to look for

satisfactory alternatives. Cells from superior cervical sympathetic ganglia, glomus cells of the carotid body and pheochromocytoma cell line [PC 12] encapsulated in semipermeable membrane have been shown to provide variable beneficial effects in rat, guinea pig and monkey models (Tresco et al 1992). A variety of other genetically manipulated cell lines have been developed to provide a source of the appropriate neurotransmitter, dopamine or acetylcholine. It may be possible to do so for other transmitters and trophic factors as well. Wolff et al (1989) genetically modified fibroblasts to provide L-dopa and demonstrated its utility for relieving symptoms in a rat model of Parkinson's disease. Immortalized cell lines have been generated by retrovirus-mediated V-myc transfer into murine cerebellar progenitor cells, by culturing cells from human megalencephaly, and by somatic-cell fusion.

Cells capable of secreting a specific neurotransmitter can be encapsulated in a nonbiodegradable polymer. This prevents immunological destruction. These encapsulated cells have been shown to survive for long time and compensate for lesion induced deficit (Tresco et al 1992). It is still too early to say whether these efforts will succeed in providing a reliable source of transplants for therapeutic purposes in humans. Continued studies are no doubt essential.

In conclusion, at the current stage of our knowledge and the experience gained from limited human trials it is obvious that neural transplantations holds lot of promise for relief to a large number of neurologically disabled individuals, who have no other hope. It is ethically permissible, not as a routine treatment but as a measure of last resort carried out by well qualified teams, under controlled conditions, in institutions with capabilities to scientifically monitor such patients for long periods. Meanwhile efforts should continue in experimental laboratories to resolve the uncertainities still associated with the procedure.

This review is dedicated to the memory of Prof. Baldev Singh who was the inspiration behind several experimental studies done by the author along with Prof. G.S. Chhina and Prof. V. Mohan Kumar, which added to our current concepts of neural transplantation. Prof. Baldev Singh was associated with pioneering advances in India in this field.

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Entero-Hypothalamo-Insular Axis Revisited

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PREAMBLE

The energy demands of the body under resting basal, active and stressful conditions are adequately and appropriately responded to in a short period of a few minutes, by glucose which constitutes a most dependable energy supply source on a short term basis, although in the long term, body adipose tissue responds to the needs for the maintenance of energy balance. Based on our collaborative studies, Bajaj et al in 1975 proposed the existence of an Entero-hypothalamo-insular axis[4]. Subsequently Bajaj (1976)[5] summarised the evidence for its metabolic role delineating neuroendocrine mechanisms involved in the regulation of energy balance. In this publication, it was specifically observed: *'The rate of glucose utilisation seems to be the set point in the regulation of entero-hypothalamo-insular axis. However, this may be so for the maintenance of energy balance on a short term basis. Adipose tissue functions as the major source of energy fuel; during starvation, glycogen stores in the human body may sustain life for less than 24 hours while energy stored as triglycerides can maintain supplies to vital organs for 30-60 days. It is therefore possible that control of triglyceride storage may be of considerable influence as a long range regulator of entero-hypothalamo-insular axis'*. The present review provides a critique of information generated during the last two decades and assess the validity and authenticity of the foregoing statement in the context of current state of knowledge.

Thus, two physiological control systems seem to operate constantly and efficiently, essentially by building sufficient carbohydrate and triglyceride reserves and by ensuring their regular replenishment and mobilisation. A complex interplay of nervous, hormonal and metabolic pathways is involved in

The review is dedicated to the memory of Prof. Baldev Singh who along with Prof. G.S. Chhina closely collaborated with the author in several experimental studies which formed the basis of the concept of entero-hypothalamo-insular axis.

this process. Our understanding of actions of insulin and glucagon, of the metabolic processes such as gluconeogenesis and glycogenolysis on the one hand and of lipogenesis and lipolysis on the other, and of the neuroregulation of secretion and action of other hormones regulating and controlling intermediary metabolism, has been facilitated by rapid developments in the fields of molecular biology and immunology. A clearer picture has therefore emerged since the time our group (G.S. Chhina, late Dr. Baldev Singh and J.S. Bajaj) published our first review of the subject in 1972[1], and followed it up with a series of publications based on additional experimental studies (Bajaj et al 1974 a, b[2,3]; Bajaj et al, 1975[4]). The present review is an attempt to establish the continuity of our early work underlying the conceptual framework of entero-hypothalamo-insular axis, with the subsequent developments in this field, finally providing an insight into the state of the art at the turn of the millennium.

HISTORICAL PERSPECTIVE

Neuroregulation of Glucose homeostasis

Prior to our studies referred to above, there seemed to be a general consensus among the investigators regarding the following key aspect of brain glucose metabolism, with the tacit assumption that:

(i) insulin was not required for utilisation of glucose by the central nervous system;

(ii) insulin did not, and indeed was not capable of, crossing blood-brain barrier (BBB), and thus produced no metabolic effects in the brain.

In contrast, entero-hypothalamo insular axis (EHI) proposed by us was a radical departure from the generally held view, and operated on the premises that:

(i) insulin affects glucose utilisation of neurones in the ventromedial (VMH) and lateral hypothalamus (LHA), thus modulating neuronal activity in these areas;

(ii) electrical stimulation of LH in the conscious rhesus monkey stimulated insulin secretion from pancreatic beta cells; and

(iii) similar electrical stimulation of VMH resulted in a decrease in pancreatic insulin secretion.

These premises, along with the demonstration of evoked responses (ER) from the VMH and LHA following stimulation of mesenteric nerve and the changes produced in the ER following acute administration of glucose and insulin, provided the essential scaffolding for our proposed hypothesis.

Entero-hypothalamo-insular Axis

The entero-hypothalamic afferents of the proposed axis were demonstrated through the evoked responses (ER) elicited from the defined regions of hypothalamus as a result of electrically stimulating mesenteric nerve in anesthetised cat, with stainless steel bipolar electrodes implanted

sterotaxically in the VMH and LHA. Mesentric nerve was stimulated by employing positive square wave pulses of 0.05 - 0.2 msec duration, and an amplitude of 5-15V (Mohankumar 1971[6]; Chhina and Bajaj, 1972[1]). From the VMH evoked responses with a prominent negative phase were obtained. In contrast, ER from the LHA almost always showed the first deflection of positive polarity followed by a negative phase. The reciprocal relationship between the state of excitatory neuronal activity in the VMH and the inhibitory activity in LHA was confirmed by the consistent observation that the negative phase of ER in the VMH corresponded with the positive phase of the evoked response from the LHA.

Intravenous glucose administration produced a potentiation of the ER from VMH; this effect was more pronounced on the negative phase and was demonstrable within one minute of glucose adminitration. In contrast, a decreased (inhibitory) response in the amplitude of both the negative and positive phases was observed from the LHA concurrently with the increase (excitatory) in the negative phase ER from VMH. Intravenous administration of insulin resulted in a sudden transitory increase in the amplitude of ER from the VMH; this lasted for about 5 minutes and was followed by a decrease in ER amplitude. The ER from LHA immediately following insulin administration showed a decrease in amplitude of both phases, with complete recovery of the response in about 5 minutes, followed by a slight potentiation of negative phase after a further lapse of 5 minutes (Fig. 1).

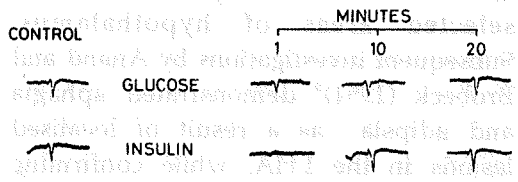

Figure 1. Effects of glucose and insulin on ERs from lateral hypothalamus on stmulation of mesenteric nerves. Glucose produced initial drop in amplitude which gradually recovered and then even increased (not shown in this figure). Insulin caused an initial short-lasting inhibition followed by an increase in emplitude (From: Chhina and Bajaj, 1972; Bajaj et al., 1975).

While these experiments provided evidence for the existence of some of the intestinal afferent pathways to hypothalamus, the afferent (effector) part of the EHI axis were elucidated by a series of experiments in normal and streptozotocin-induced diabetic rhesus monkeys as reviewed in the following paragraphs.

Hypothalamus: Then and Now

Chronologically, the credit for demonstrating the role of hypothalamic mechanisms in the regulation of energy balance must be given to Heterington and Ranson who in 1940, produced obesity in the rat by lesions confined to VMH. Subsequently, Brobeck et al (1943)[7] showed that the hypothalamic obesity was due to hyperphagia. The functional role of hypothalamus through autonomic nervous system received major attention culminating in the award of Nobel prize

to Walter Rudolph Hess in 1949 for his poineering studies on hypothalamus and autonomic function. Such a recongnition provided further impetus to those involved in the study of leisons in selected areas of hypothalamus. Subsequent investigations by Anand and Brobeck (1951)[8] demonstrated aphagia and adipsia as a result of localised lesions in the LHA, while confirming hyperphagia and obesity as a result of lesions restricted to the medial portion of the hypothalamus. They proposed the terms 'feeding' and 'satiety' centres to describe defined areas in the lateral and ventromedial hypothalamus, respectively.

Bernardis and Bellinger in a recent review (1993)[9] concluded that several of the changes originally ascribed to lesions in LHA might be due to a reduction in food intake, while a number of metabolic alterations were possibly due to a 'true' lesion effect, involving profound changes in glucose metabolism such as glycolysis, glycogenesis and gluconeogenesis. It is argued that the rats with LHA lesions regulate their body weight 'set' point in a primary manner, and not because of the destruction of a feeding centre. In the early stages of the syndrome, catabolism and running activity were enhanced, and so was the activity of the sympathetic nervous system as shown by increased excretion of norepinephrime which tends to normalize after about 4 weeks. Such observations provided the requisite interface between the feeding behaviour and the autonomic activity, both regulated by the hypothalamus.

Additional data on VMH lesions provided a striking complementarity and a cohesive conceptual matrix. In addition to hyperphagia the VMH-lesioned rats show hyperinsulinemia. Furthermore, in the first phase following VMH lesion, rats are hypersensitive to insulin with exaggerated response to small doses. In contrast, as obesity becomes manifest, VMH-lesioned rats become insulin resistant with a decrease in sensitivity and responsiveness of tissues such as liver and muscle to the administration of hormone (Penicaud et al, 1986)[10].

There is progressive development of insulin resistance in the muscle after the lesion of the VMH. Six weeks after the lesion, the muscle of the lesioned animals utilised less glucose than those of controls. Simultaneously, there was a transient insulin hypersensitivity in the white adipose tissue, wherein glucose utilisation was increased more than two-fold after one week but returned to normal in six weeks. This, together with hypersecretion of insulin, possibly contributed to the increase in body fat mass by redirecting glucose towards the adipose tissue (Penicaud et al, 1989)[11].

In our studies, different hypothalamic areas, namely the VMH, LHA, preoptic, posterior hypothalamus and mamillary body were stimulated through stereotaxically implanted electrodes in conscious male rhesus monkeys. A significant decrease was observed in blood glucose following the VMH and the preoptic area stimulation. An opposite response was obtained from LHA and posterior hypothalamus. There was a

generalised significant increase produced in serum free fatty acids, but triglycerides and cholesterol remained largely unaffected (Bajaj et al, 1974a)[2].

A 4-to 6-fold increase in circulating immunoreactive insulin was observed following the LHA stimulation (Fig. 2). An opposite response was obtained from the VMH (Fig. 3). Thus, insulinogenic and insulinoprival responses were obtained from feeding and satiety centres, suggesting a significant role of these areas

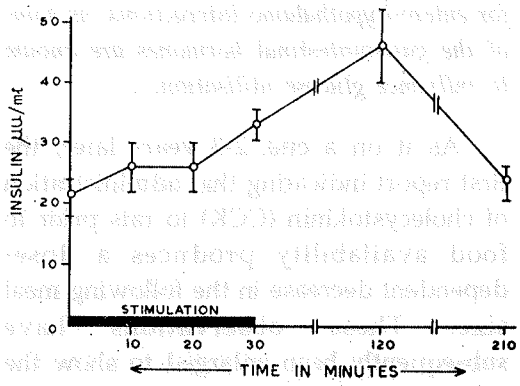

Figure 2. Effect of electrical stimulation of lateral hypothalamus on serum insulin.

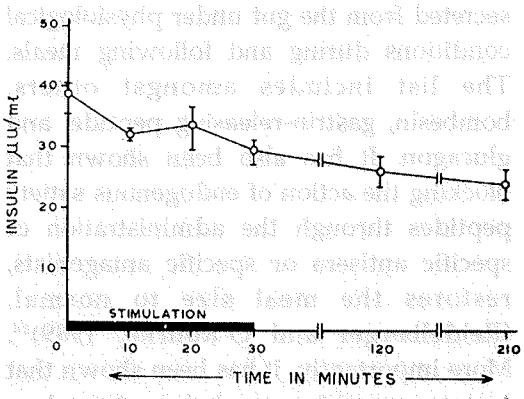

Figure 3. Effect of electrical stimulation of ventromedial hypothalamus on serum insulin.

in insulin regulation (Chhina and Bajaj, 1972[1]; Bajaj et al, 1974 a)[2]. The growth hormone and cortisol release were facilitated by the stimulation of the LHA and the VMH (Bajaj et al, 1974 a)[2]. The endocrinal responses from preoptic area resembled that of the VMH. The posterior hypothalamus and mamillary body showed trends which were common to both the LHA and VMH (Garg et al, 1983)[12].

Electrical stimulation of the VMH, in streptozotocin-induced diabetic, conscious male rhesus monkeys, significantly increased growth hormone, and decreased blood glucose. Serum insulin, free fatty acids, triglycerides and cortisol were largely unaffected, in contrast to the normal control animals where the insulin level showed a significant decrease. Cortisol and free fatty acids increased significantly by the VMH stimulation. None of the biochemical parameters showed any significant change at any time following the electrical stimulation of the parietal cortex. Thus the VMH stimulation did not alter the diabetic syndrome drastically. It also did not prevent the changes in metabolism seen after VMH stimulation in control animals (Garg et al, 1980)[13]

Insulin and Hypothalamus

Three years after the postulation of entero-hypothalamo-insular axis, the first supportive evidence for the role of insulin in CNS came from the studies of Jessie Roth's group (Havrankova et al 1978 a,b)[14,15] who demonstrated the presence

of insulin in the rat brain, with the concentrations in the hypothalamus being 2-3 fold higher than in other regions of brain excepting olfactory bulb[14,15]. Likewise, insulin receptors, with the largest population in the hypothalamus, were convincingly documented. Subsequently, expression of proinsulin messenger RNA by neurones (but not glial tissue) isolated from neonatal rabbit brain was documented (Schechter, 1988)[16]. A similar proinsulin mRNA expression was also demonstrated in neuronal cultures (Schechter et al 1990)[17]. Interestingly proinsulin mRNA expression was localised only to the neurones in the periventricular hypothalamus, an area suggested to have a role in feeding behaviour.

Thus, the earlier and traditional belief suggesting brain as an insulin-insensitive tissue, which was directly challenged and rebutted by the experimental evidence based on our collaborative studies (cited above), has now been substantially repudiated and our contention regarding metabolic effects of insulin on neurones in the hypothalamus, largely validated and accepted Indeed, insulin is recognised as an afferent CNS signal which regulates normal energy balance; although the contention whether it is synthesised *de novo* in brain, or is largely transported from plasma through blood brain barrier, remains a matter of ongoing debate.

Satiety hormones and peptides

While discussing the role of gastrointestinal hormones as possible modulators of neuronal signalling and as regulators of gastrointestinal blood flow, motility and absorption (Bajaj, 1976)[5], a reference was made to our observation regarding an increase in the circulating levels of gut GLI (Glucagon-Like-Immunoreactivity) following VMH stimulation in conscious restrained monkeys. *It was further observed that: '... no other studies are available regarding the alterations in gastrointestinal hormones following ablation or stimulation of VMH and LHA. It is likely that when the results of such studies are made available in the future, these may provide further evidence for entero-hypothalamo interactions, as some of the gastrointestinal hormones are known to influence glucose utilisation....'.*

As if on a cue, 2-3 years later, the first report indicating that administration of cholecystokinin (CCK) to rats prior to food availability produces a dose-dependent decrease in the following meal size. These observations have subsequently been enlarged to show the effect on meal size of a large number of gut peptides following exogenous administration. These peptides are secreted from the gut under physiological conditions during and following meals. The list includes amongst others, bombesin, gastrin-releasing peptide, and glucagon. It has also been shown that blocking the action of endogenous satiety peptides through the administration of specific antisera or specific antagonists, restores the meal size to normal. (Reidelberger and O'Rourke, 1989)[18]. More importantly, it has been shown that satiety peptides modulate impulses through vagal afferents, in addition to

their effect through receptors in the brain. Such afferent neuronal information passes through the brainstem to hypothalamus where it is processed and integrated with diverse neuroendocrinal-metabolic cues.

Long-term regulation of energy balance

While the role of EHI axis during the last quarter of century since the term was first coined, has been further clarified, expounded and validated, there is no doubt that it largely operates as a short-term control mechanism. Likewise, even though the satiety peptides produce a demonstrable decrease in meal size when administered immediately preceding a meal, their repeated administration does not alter body weight. This is in direct contrast to the observation of aphagia and weight loss which follows the lesions of LHA in the rat. Additional cues, through alternate neurohormonal mechanisms, must therefore operate, aimed at maintaining constancy of body weight on a long-term basis.

It is in this context that the information during the last five years has been truly phenomenal. Essentially, it deals with two interrelated operational systems:

(i) Leptin-insulin system and

(ii) Agouti-melanocortin system

There is evidence to suggest a cross-talk between these two systems.

(i) Leptin-insulin system:

Ob and db genes and their products serve as ligand and receptors, respectively, for a central pathway controlling and regulating satiety state and metabolic rate.

The recessive obesity (ob) single gene mutation in mouse produces marked obesity and results in a type of diabetes resembling human NIDDM (Friedman and Leibel, 1992)[19]. The mouse ob gene was subsequently isolated by positional cloning (Zhang et al, 1994)[20].

These ob/ob mice, in addition to being severely hyperphagic, also have a low body temperature thus combining increased energy intake due to excess appetite with reduced energy expenditure, leading to explosive weight gain. Additional features, such as high circulating levels of glucose, insulin and cortisol, tend to indicate a wider role of leptin in mammalian physiology. Furthermore, these animals are infertile suggesting involvement of leptin in hypothalamic pituitary-gonadal axis (Cameron, 1996)[21]. All of these features are reversed by administering mice leptin, the product of ob/ob gene (Campfield, 1995)[22]. Not only is the fertility restored in ob/ob mice following leptin administration, such a treatment also accelerates the outset of reproductive function in normal rodents (Chehab et al, 1997)[23]. Thus plasma leptin may act as a signal of nutritional state, laying down the hierarchial priority of self-survival (feeding behaviour) over species survival (reproductive behaviour).

Fasting and exercise decrease ob gene expression while feeding increases expression in adipose tissue ob mRNA.

Interestingly, these changes parallel insulin levels in circulation (Becker et al, 1995)[24]. There is substantive evidence to indicate that the adipocyte-specific hormone leptin, the product of the ob gene, regulates mass of adipose tissue through effects on satiety and by regulation of energy expenditure. In contrast to the satiety peptides such as CCK, the repeated administration of leptin induces weight loss by decreasing food intake and/or increasing energy expenditure (Levine et al 1996)[25]. Leptin acts through a single transmembrane domain receptor gene which is located on chromosome 4 in the mouse. The long form of the leptin receptor is primarily expressed in hypothalamus, while the short forms of leptin receptor are expressed in other areas of the brain, as well as in several other tissues (Lee et al 1996)[26]. In situ hybridization with antisense riboprobe shows strong expression in ventromedial, paraventricular and arcuate nuclei of hypothalamus (Mercer et al 1996)[27]. In the mouse, homozygous mutation in gene encoding leptin receptor causes obesity, hyperphagia and reduced energy expenditure. A recent publication (Montague et al, 1997)[28] describes the association of early-onset obesity with a mutation in the leptin gene. As was only to be expected, a subsequent publication followed early this year, describing for the first time human obesity and pituitary dysfunction as a result of a mutation in the human leptin receptor gene (Clement et al 1998)[29]. The authors conclude: ' Our results indicate that a functional leptin receptor is required not only for the regulation of body weight but also for sexual maturation and for secretion of growth and thyrotropic hormones. Leptin is therefore a critical link between energy stores and hypothalamic pituitary functions in humans'.

Hormonal regulation of Leptin

Circulating leptin levels remain unchanged following food intake in human subjects, suggesting a lack of effect of postprandial hyperinsulinemia on circulating leptin (Considine et al 1996)[30]. Likewise combined glucose and tolbutamide challenge, which is a major stimulus for β-cells, did not produce any significant change in basal leptin levels in lean insulin-sensitive, lean insulin-resistant and obese insulin-resistant male subjects with normal glucose tolerance (Segal et al 1996)[31]. However, small but significant gender difference may be present as shown in the study of Kennedy et al (1997)[32] wherein a 20% rise in basal leptin was observed in the females subjected to hyperinsulinemic-euglycemic clamp while no effect was demonstrable in male subjects under similar experimental conditions.

In contrast to in vivo studies in the human, in vitro studies using differentiated human adipocytes, a stimulatory effect of insulin on leptin secretion was consistently demonstrated (Wabitsch et al 1996)[33]. The reasons for differential in vivo and in vitro effects, and their physiological significance if any, need further elucidation.

In summary, leptin has a dual regulatory function in human physiology,

affecting feeding and reproductive behaviour. During the period of weight maintenance, when energy intake equals energy expenditure, circulating leptin levels reflect total body fat mass. However, during a significant alteration in body weight, leptin levels serve as sensor of energy imbalance: increase in body fat-mass results in enhanced leptin secretion from peripheral adipocytes while even a short term fasting decreases leptin secretion, with circulating leptin levels declining to nearly 30% of initial basal levels. Unlike marked changes in serum leptin, CSF leptin is only moderately increased in obese subjects and the CSF leptin/serum leptin ratio decrease logarithmically with increasing body mass index.

Mechanism of Leptin Action

The regulatory pathways underlying central action of leptin have recently been reviewed and summarised (Flier and Maratos-Flier, 1998)[34]. At the onset of obesity, with increasing triglyceride storage in adipocytes, leptin secretion is enhanced, resulting in increase in both the peripheral and CSF circulating levels of leptin. In the CNS, leptin acts on receptors in the arcuate nucleus of the hypothalamus, and inhibits secretion of neuropeptide Y (NPY). NPY is a 36 aminoacid polypeptide synthesised by neurones in the CNS as well as in the peripheral nervous system. In the CNS, NPY synthesis is localised to neurones in the arcuate nucleus of hypothalamus and its subsequent release is through their axons in the paraventricular nucleus. The production of NPY (in the arcuate) and its secretion (in PVN) is affected by the state of energy balance. Increased levels of leptin, in response to increased adipocyte mass, suppress appetite and reduce food intake by inhibiting NPY synthesis and release, thereby constituting a major regulatory mechanism.

It is of considerable interest to note the role of NPY as a common regulatory pathway, shared both by leptin and insulin. Like leptin, insulin also is a peripheral adiposity signal to CNS. Such a contention is supported by a large dataset cumulated over the last few years: basal plasma insulin levels show marked increase in obesity as well as in those states characterised by a reduction in every expenditure; there is a dose-dependent entry of peripheral circulating insulin into the CNS; neurones express high concentration of insulin receptors in hypothalamic areas known to regulate feeding behaviour; and finally, the most significant effect of small doses of insulin following either central or peripheral administration (so long as hypoglycemia is avoided) is to produce a marked reduction of food intake and a state of negative energy balance resulting in weight loss. That such an effect is mediated through the VMH was shown by an increase in food intake following acute injection of insulin antibodies into the VMH of rats during dark portion of day/night cycle (to time it with the natural diurnal eating pattern in the rat). More recently, McGowan et al (1990)[35] have reported a more chronically increased food intake and rate of weight

gain following repeated chronic infusion of insulin antibodies into ventromedial hypothalamus in the rat.

There is now evidence to suggest that like leptin, insulin also mediates its effects through NPY. Inhibition of hypothalamic NPY gene expression by insulin has been reported (Schwartz et al, 1992)[36]. Complementary data, supporting insulin - NPY interaction in the hypothalamus has been provided in both the spontaneously diabetic (non-insulin dependent) BB and in streptozotocin - induced insulin-dependent animals rat (Abe et al, 1991)[37]. In the latter, with marked insulin deficiency, augumentation of hypothalamic expression of preproNPY mRNA was demonstrated (Sahu et al 1990)[38]. Both the NPY and its mRNA showed increased hypothalamic levels in insulin deficient animals, and both were normalised following systemic insulin therapy. In this connection, it may be of interest to recall our data regarding bipolar EEG recording in the normal and streptozotocin-induced monkey which showed a slower activity in the ventromedial hypothalmus, returning to normal after insulin administration (Bajaj, 1976)[5]. It is therefore justified to suggest that both the EEG changes in VMH and hyperphagia in the diabetic monkey, were possibly due to augumentation of NPY levels in the arcuate-paraventricular nuclei. The changes were reversed following systemically administered insulin therapy. Both insuln and leptin are secreted in increased amounts in obesity, and both act through central receptors involving NPY system.

(ii) Agouti-Melanocortin system

Recent studies based on cloning and characterization of a number of mouse obesity genes (Ob and db genes have been discussed in detail above), such as agouti, tub and fat have provided mutant mice which are suitable models of moderate, slow-onset mouse obesity, that may be closer to adult-onset obesity (and diabetes) in the human (Naggert et al 1995[39]; Noben-Trauth et al 1996)[40]. Agouti was the first amongst these obesity genes to have been cloned (Bultman et al, 1992)[41]. This gene is normally involved in coat colour regulation in mice. It encodes a 131-aminoacid molecule which exerts a paracrine action on melancocytes. Dominant mutations at the agouti gene result in mice, with predominantly yellow fur, characterised by progressive obesity, hyperinsulinemia, peripheral insulin resistance, impaired glucose tolerance, decreased thermogenesis and mild hyperphagia (Liebel et al, 1997[42]; Michaud et al, 1997)[43]. As agouti modulation of pigmentation is mediated through the fact that the agouti protein competitively inhibits αMSH binding to melanocyte melanocortin-1 receptor (MC1-R), studies were initiated to investigate whether a similar antagonism at other MC-receptors may form the basis of the metabolic syndrome in agouti mutant mice. In addition to MC1-R, there are four other members of the MC-R family: MC2-R is the ACTH receptor; MC3-R is found in the hypothalamus and limbic system; MC4-R is widely expressed in may regions of the brain, and MC5-R is expressed in a large number of body

tissues. As both MC3-R and MC4-R are expressed in the VMH, it was suggested that agouti antagonism at these sites may explain hyperphagia. Indeed, targetted disruption of the murine MC4-R (Husgar et al, 1997)[44] and the recent data from a family study in Quebec (Chagnon et al 1997)[45] tend to support such a hypothesis.

Several studies indicate that there is a significant cross-talk between the leptin and agouti-melanocortin signaling pathways. Leptin, while inhibiting secretion of NPY, increases the release of (MSH and at the same time decreases the secretion of agouti-related peptide (AGRP) which is an antagonist of αMSH at melanocyte melanocortin-4 (MC4) receptor and is expressed in several regions of the brain. It is the MC4 receptor which is 'feeding-inhibitory'. The net output from the feeding inhibitory MC4 signalling pathway located in the ventromedial hypothalamus may be determined by the ratio of agonist (αMSH) and the antagonist (AGRP) at the MC4 receptor neurone. Leptin alters the ratio in favour of αMSH/ AGRP by increasing αMSH, and reducing AGRP secretion, in the ventromedial hypothalamus thus suppressing food intake.

It is abundantly clear that for the first time, it is possible to visualize molecular basis of signalling of 'satiety' neurones, described by Anand and Brobeck nearly 50 years ago, and followed by our subsequent studies resulting in the concept of entero-hypothalamo-insular axis, and its role in energy balance on a long term basis.

OREXINS : The Feeding Molecule(s)

The mechanisms of molecular signalling in the ventromedial hypothalamus and of the agouti-leptin-insulin cross talk in and through the neurons and synapses, have been amplified and properly 'tuned' to optimise the noise: signal ratio. In contrast, the 'silence' of the lateral hypothalamus remained intriguing till early this year when modern tools of molecular biology were used to explore this hitherto silent area. The result is an exciting and most fascinating development.

Using the modern methods of molecular recognition, Sakurai et al (1998)[46] discovered two key hypothalamic neuropeptides, now named Orexin A and B (Gk. *orexis*, appetite) which are located in the 'feeding' centre of lateral hypothalamus. Perhaps, recognising that the original observations of Anand and Brobeck wherein ablative lesions produced in the rat lateral hypothalamus resulted in aphagia and weight loss were true and valid, Sakurai et al set upon the task to find neuropeptide(s), if any, underlying the behavioral and metabolic changes which result from such lesions. Expressed sequence tags (ESTs) available from public databases, with sequence homologies to known G protein-coupled cell-surface receptors (GPCRs) were used for screening with human brain cDNAs to obtain full length cDNAs and fifty stable transfectant cell lines were generated as a result. Each of these cell lines expressed a distinct orphan GPCR cDNA. Crude peptide extracts from rat

brain were tested on the transfected cells for GPCR agonist activity by measuring increase, if any, in cytoplasmic Ca^{++} level, a common response to G protein receptor activation.

A major active peptide fraction was isolated, purified to homogeneity in four steps by HPLC, sequenced, and its structure established by mass spectrometry. The resultant peptide, called Orexin A consists of 33 aminoacids (MW = 3562 Da). A second peak of activity was separated and named Orexin B (MW = 2937 Da). Orexin A cDNA was made by reverse transcriptase treatment of rat brain mRNA followed by PCR. The structure of prepro-orexin gene has been determined, and it has been located on chromosome 17q21. Both the 33 aminoacid Orexin A and 28-aminoacid Orexin B are encoded by a single mRNA transcript. Also identified were two Orexin receptors. Orexin A receptor (Ox1R) showed a 26% identity with NPY. While Orexin A was a specific high affinity agonist for Ox1R stimulation, a second receptor Ox2R with 64% identify to Ox1R and high affinity binding of Orexin B has also been sequenced. Ox2R also binds Orexin A.

To localise Orexin expression within the CNS, in situ hybridization and immunohistochemical analyses in the rat brain was performed. It showed the presence of prepro-orexin mRNA bilaterally and symmetrically in the lateral and posterior hypothalamic areas and the perifornical nucleus. It is noteworthy that no signal was detected in neurons of paraventricular, ventromedial or arcuate neclei, areas which are known to contain neuropeptides such as NPY associated with food consumptin, which are leptin sensitive. Impressed by the 'striking localisation' of Orexin containing neurons in the lateral hypothalamus and some of its adjacent areas, and none in the VMH-Arcuate-PVN area, Sakurai et al investigated the effect of Orexin administration through preimplanted indwelling catheters. Within one hour of intracerebroventricular administration of Orexin A bolus, food consumption was stimulated in a dose-dependent manner. Human Orexin-B administered similarly in the rat also augumented food intake significantly but the duration of its action was shorter. These actions of Orexin were similar to, albeit of lesser order of magnitude, than those observed following NPY administration.

Epilogue

In concluding our paper published in 1976 (Bajaj, 1976)[5], it was stated:

*'Obesity resulting from a functional disorder of the hypothalamus remains a remote possibility. A clear delineation of possible alterations in the normal physiological control mechanisms involved in the EHI axis is likely to produce better insights in the diagnosis as well as management of diabetes mellitus. The possible therapeutic effects of yoga may be mediated through alterations either at the level of sensory inputs (entero-hypothalamic) or at the level of central receptor mechanisms (Bajaj, 1976 a)[47]. **Future development of specific neuropharmacological agents, modifying EHI axis remains a distinct***

therapeutic possibility in the management of obesity and diabetes mellitus'.

It is gratifying to note that nearly a quarter century later, in concluding the discussions of their paper (Sakurai et al, 1998)[46] amplify the above views, using their own work and citing other observations, eventually suggesting similar, if not identical, future possibilities. The concluding paragraphs of their discussion are reproduced below:

'Recent studies continue to reveal the molecular basis for the role of periventricular/medial hypothalamic regions in energy homeostasis, e.g., ventromedial nucieus, arcuate necleus, and paraventricular nucleus. Neurons containing neuropeptides such as NPY (Bing et al, 1996)[48], melanocortins (Jacobowitz and O'Donohue, 1978)[49], glucagon-like peptide-1 (Shughrue et al, 1996)[50], and galanin (Warembourg and Jolivet, 1993)[51], as well as the leptin and melanocortin-4 receptors (Mountjoy et al, 1994[52]; Tartaglia et al, 1995)[53] are abundant in one or more of these periventricular/medial hypothalamic regions. In contrast, few neuropeptides have been described to be produced chiefly in the lateral hypothalamic regions. Other than the orexins, we are aware of only one distinct neurotransmitter that is specifically produced in the lateral hypothalamus: melanin concentrating hormone (MCH) has been localised in the zona incerta and the lateral hypothalamic area (Bittencourt et al,. 1992)[54]. Intriguingly, MCH was recently reported to stimulate food intake upon central administration. Moreover, MCH mRNA is up-regulated in ob/ob mice and by fasting in wild-type mice (Qu et al, 1996)[55]. It will be important to investigate further the possible interplay of orexin with this and other positive (e.g., Agouti-related protein, NPY, galanin, and opioids) and negative (e.g., leptin, melanocortins, corticotropin-releasing factor, glucagon-like peptide-1, and cholecystokinin) regulators of energy balance both within and outside the central nervous system (Arase et al, 1988[56]; Rosenbaum et al, 1997)[57]

A decline of blood glucose levels can signal the initiation of food intake (Oomura, 1980)[58]. The lateral hypothalamic area contains glucose-sensitive neurons that are activated by glucopenia and thus implicated in the positive short-term regulation of feeding and energy expenditure. It is tempting to speculate that all or some of the orexin-containing neurons may be glucose-sensitive, or that they may receive stimulatory projections from glucose-sensitive neurons. Future experiments will also determine whether orexins have additional actions relevant to nutritional homeostasis, such as effects on the regulation of systemic energy expenditure, secretion of metabolic hormones such as insulin, and ultimately, the regulation of body weight.

The present discovery of orexins and their receptors may provide a novel molecular basis for the role of the lateral hypothalamic areas in the regulation of feeding behaviour. It is clear, however,

that the definitive assignment of physiological roles for orexins requires further pharmacological as well as molecular genetic investigations. ***Nevertheless, pharmacological intervention directed at the orexin receptors may prove to be an attrative avenue toward the discovery of novel therapeutics for diseases involving disregulation of energy homeostasis, such as obesity and diabetes mellitus.***

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It is immaterial that Sakurai et al (1998)[46] do not cite Bajaj (1976)[5]. What is important in science is the validation of original observations and the postulated hypotheses. Some of us including B.K. Anand, G.S. Chhina and myself are fortunate to see this happen in our own life time. Unfortunately, Sakurai's paper was published in the February 20, 1998 issue of the journal, Cell. Dr. Baldev Singh died on February 2, 1998 without knowing the work on Orexins !

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