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Story of Muscarinic Receptors, Alkaloids with Muscarinic Significance and of Muscarinic Functions and Behaviors

Alexander G Karczmar*

Research Service, Edward J. Hines VA Hospital and Department of Pharmacology, Loyola U. Medical Center, IL, USA

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Abstract

Karczmar AG. Story of Muscarinic Receptors, Alkaloids with Muscarinic Significance and of Muscarinic Functions and Behaviors. Annu Rev Biomed Sci 2009;11:T1-T50. This review of the studies of the muscarinic receptors, their synaptic activities and their functional and behavioral roles will begin with the history of the research of the autonomic and central nervous systems and their transmitters, the development of the notion of the receptor, and the tale of the significance of muscarine and other alkaloids as well as of organophosphorus (OP) anticholinesterases for these studies; we will then segue into the modern status of muscarinic receptors and of their functional and behavioral expression.

Keywords: muscarinic, cholinesterase, cholinergic, nicotinic, curare, atropine, behavior

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* **Correspondence**
Alexander G Karczmar, 1830 North Lincoln Ave, Chicago, IL 60614, USA. E-mail: karczmar@sbcglobal.net

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1. Early Story of the Autonomic and Central Nervous System

Available histories of the autonomic and the central nervous system (see, for example, Castiglioni, 1947; Brazier, 1959; Pick, 1970; Robinson, 2001) begin with the story of Greek and Roman investigators; this is not a case of Western chauvinism, as actually the "real" anatomical studies were not carried out in India, Persia and China, and only to a limited extent in Egypt. Thus, while the Indian Vedic (5000 B. C.) and postvedic as well as the Persian and Buddhist medicines were concerned with a number of specialties, including geriatrics, psychiatry and surgery, and the Indian surgeon Shushruta (about 600 BC) insisted on the need of dissections and introduced surgery of the prostate and the intestine, and cosmetic surgery including rhinoplasty, the anatomy as we understand the term was not a subject dealt with by ancient Indian, Tibetan or Persian medical men (see Rajgopal *et al.*, 2002; Zimmer, 1951; Shoja, 2007). Similarly, while Chinese were concerned with healing and medicine before and after the 3000 B. C. treatise, "Nei Ching" edited on behalf of the Emperor Huang Ti (Huang & Jing, 2004), anatomy was not considered in their investigations. Nor were Egyptian physicians and sages concerned with systematic study of anatomy, as active as they were in several areas of medicine and treatment, including skull trepanning (Silverburg, 1966), although the so-called Edwin Smith Surgical Papyrus dating from 1700 B. C. described the brain, some details of its anatomy and the meninges. Altogether, the Egyptians could not consider the brain very highly, since they discarded, not preserved, the brain during the burial. The reason for this neglect and for the difference between the western on the one hand and the Egyptian and Asiatic on the other approaches to medicine is due to the difference in their respective notions of medicine and anatomy; the European approach being practical and pragmatic, while Egyptian and Asiatic approaches being esoteric, spiritual and metaphysical (Matuk, 2006).

On the other hand, Greek and Roman philosophers were concerned with the brain early, even before the Greek and Roman investigators became involved in the anatomical study, in the modern sense of the term, of the brain and the nerves. Thus, Plato (427 – 347 B. C.) believed that the brain is the site of "intelligence" and of mental processes, although Aristotle (384-322) who, in contradistinction to Plato was not only a philosopher but a dissector of animal bodies, preferred the heart as a center of emotions and thoughts, the brain serving only to cool the heart. They also had some ideas as to the site and nature of the soul [1]. Peripheral autonomic nerves and the network of nerves emanating from the brain and the spine were described by the Greek anatomists (they dissected the human bodies) of the fourth and third Century B. C., such as Herophilus and Erasistratus; indeed, the earlier Greek investigators considered the nerves to be tendons, or parts of the arteries. Actually, Herophilus (335 - 280 B. C.) and Erasistratus (304 - 250 B. C.) described the ventricles of the brain, and Erasistratus thought that they contained the intellect whose "spirits" flow down the nerves to the muscles, activating the latter; yet, the brain itself had no command of

[1] The story of the soul, of the "I" and of self-awareness is an immense subject that is distinct from that of human behaviors and of cognition; it is generally outside the scope of this article, except in the specific case of Nancy Woolf's hypothesis of the muscarinic contribution via certain cortical cells to the "I" (see below). Actually, many of the investigators of the functions of the nervous system and of behaviors, referred to in this article, were as well concerned with the soul or the "self", but only short references are made in this article to this particular area of their work (see also Karczmar, 2007).

the body! Notabene, their predecessor and "the father of medicine, Hippocrates (460 - 377 B. C.) did not seem to describe human or animal anatomy.

It took some 175 years before substantial progress was made in anatomy and medicine. The Romano-Greek medical man, Galen (129 - 200 or 217 A. D.) [2], the scion of a rich patrician family of Pergamon lived and studied in Pergamon, Alexandria and Rome, and he travelled as a friend and physician of Roman leaders and emperors, throughout Asia. He was not allowed to dissect human bodies by the Roman law, but he dissected many species of animals, including apes. Following to an extent Erasistratus, Galen spoke, like his predecessor, of "vital spirits" that travelled in blood, were "cooked" in the heart and mixed with the air in the lung, and then were transported via the vessels of the base of the brain (today known as rete mirabilis) to the ventricles, whence they would be driven by brain's pulsations, into hollow nerves, carrying sensations and emotions (Galen assumed, wrongly, that the rete mirabilis of the apes exist in the humans); and, similarly to Erasistratus, Galen thought that the human intelligence is lodged in the empty ventricles. Galen wrote also about the autonomic nervous system, as he illustrated "swellings" of the cranial nerves that corresponded to superior and nodose ganglia; these "swellings"were, unmistakably ganglia. He also described, among peripheral nerves, a nerve that probably corresponded to the vagosympathetic trunk and its rami communicantes, without using this terminology (see Karczmar, 1986; Zimmer, 2004). Galen (see the Paris edition, 1528 of Galen's work) was a most prolific writer (he wrote generally in Latin), he introduced a number of general medical concepts such as his theory of humors as controlling human health and disease, and he described the use of many herbs and plants, referred to ever since as galenicals. His work is quoted through two millennia.

Indeed, Galen's findings were not questioned in the Western world till the advent of the Renaissance investigators (see below), even though the Arab medicine, as represented by Razi, Avicenna and Zuhr (Avenzoar) – and the Jewish medical man and philosopher, Maimonides or the Second Moses – progressed in the IXth, Xth and XIth Century beyond Galen's knowledge: their findings were available in Europe via the translations of the materials in question in Spain and in Italy (see Durant, 1950), however, these findings somehow did not enter the world of Western medicine till the days of Renaissance (see Rothschuh, 1973; Park, 1990).

Ironically, the Arab doctors and scientists were well acquainted with Greek scientists, physicians and philosophers, including Galen. In fact, the great physician Abu Bekr Muhammad al-Razi (Razi or Rhazes; 844-926) corrected many errors in anatomical findings of Galen and in Galen's concepts, as did the greatest Islami scientist, physician and philosopher, Avicenna (Abu Ali al-Husein ibn Sina; 980-1037; see Park, 1990). They promoted autopsies and dissections and experimental approach to medicine and to the testing of remedies, and their anatomical illustrations show clearly their knowledge of brain, the spinal cord and their radiations, including motor and autonomic innervations (Sarton, 1927-1948; Abdel-Halim, 2005; Jacquart, 2008; see also Wikipedia, 2009).

The matters started moving in the Renaissance era. In that era and then in the seventeenth and subsequent centuries the pertinent investigators frequently described both the central and the peripheral – including what was referred later as the autonomic - nervous system. Not all of them were professional anatomists or doctors: between 1510 and 1511 the great Leonardo da Vinci (1452-1519) dissected, in association with doctor Marcantonio della Torre, human bodies in the Hospital of Santa Maria Nuova in Florence; he described and illustrated in detail the human nervous system, including the central nervous system, its ventricles and its connections with the olfactory and optical nerves, as well as the peripheral nervous systems. While his work constituted a significant advance over that of Galen and the Arab doctors, Leonardos' work and illustrations did not enter the ken of the medical world of his era, perhaps because his notes were made in a code (he wrote

[2] Galen's of Pergamon first name is usually given as Claudius; this is, probably, a Reinaissance fabrication. He is sometimes referred to as Aelius Galenus, Aelius being perhaps a patronymic.

from right to left, and in his writing the sequence of letters in a word was reversed), and also because the illustrations were published only after Leonardo's death in Paris [3].

The physician, anatomist and dissector Andreas Vesalius (1514-1564; see O'Malley, 1964) was born in Brussels, studied in Padua and Bologna, and was the court physician to Charles V and Philip. He travelled extensively with both of them and on his own, and he died in a ship wreck of the coast of Greece. He did study carefully Galen's works, indeed, he may have plagiarized Galen's work on the nervous system (see Saunders & O'Malley, 1973). He also criticized some of Galen's findings and concepts, and he noticed that some of Galen's findings concern animals rather than humans. He described and illustrated the skeleton and many organs, including the brain and the nerves. He posited that nerves are not hollow, as maintained by many of his predecessors, and demonstrated that, contrary to Aristotle and many others, nerves do not originate from the heart, but from the brain and the spinal cord; finally, he described the corpus callosum, the caudate nucleus, the lenticular nucleus, the globus pallidus, the putamen, the pulvinar and the cerebral peduncles. And he maintained that the brain and the nerves are the site of the human intelligence. But he refused to commit himself to defining the site and the nature of the soul, probably out of fear of antagonizing the Church if he did so.

Vesalius' XVIth Century followers such as Charles Estienne and Bartolommeo (or Bartolomeo) Eustachio improved on Vesalius' and Galen's findings as they illustrated the separation between the vagus and sympathetic nerves (see Pick, 1970; Karczmar, 1986), again without using the modern terminology. And W. Croone (published posthumously in 1852) and Albrecht von Halle (1706-1777) followed Galen's belief that spirits or "spirituous liquids" descend down the tubular nerves and initiate the effector function. And, the great mathematician and philosopher, Rene Descartes (1596-1650), who was actually a student of medicine and anatomy shared their concept of the "spirituous liquids". During the subsequent century two celebrated philosophers and medical men, the Dutchman Hermann Boerhave (1688-1738) and the Frenchman Julien Offroy de la Mettrie (1709-1751) contributed more to the theories than to the biology of brain function (thus, Boerhave opined that the brain serves to purify the blood…).

On the other hand, C. Reid, F.-P. Petit, Raymond Vieussens (1641-1715) and, particularly Thomas Willis (1621-1675), the great French and English anatomists and doctors contributed to the real knowledge of the nervous functions, as they described in detail the sympathetic trunk and the cranial nerves, including the vagus (Willis used the term "vagus" although this author is not sure as to who was the first investigator to use this term) and Petit (1727) showed that the "intercostal" chain is of subcranial rather than cranial origin; he also believed that the intercostals carry "the animal spirits" into the eye. Willis was the first to establish, in the dog, vagal effects on the heart and lung (Willis, 1664; see also Nordenskiold, 1936 and Karczmar, 1986), and he studied the brain of many animal species, as well as dissected many adult (and some juvenile) brains. He described the spinal cord, the cerebral cortex, the medulla, cerebellum, corpora striata (which he referred to as the 'sensorium commune'), corpus callosum and the cranial nerves (see Dewhurst, 1982). He demonstrated that the nerves are not hollow and he opined that the "superior faculties" of the human are located in the brain and expressed via animal spirits carried in the nerves. Altogether, Willis, who coined the term 'neurology', was a great anatomist (he also achieved fame and wealth as a doctor; see Dewhurst, 1982 and Zimmer, 2004).

The Swiss polymath, Albrecht von Haller (1707-1777) was regarded by his contemporaries as a great anatomist and physiologist, yet his contributions to the study of the brain and peripheral nervous

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[3] Some 120 years after Leonardo drew his human anatomies, another great painter depicted the dissected human bodies. He was Rembrandt van Rijn (1606-1669) who painted, in 1632 and 1656, "Anatomy Lesson of Dr. Nicolaes Tulp" and the less well known "Anatomy Lesson of Dr. Jan Deijman", respectively (in Netherlands, dissections of criminals were permitted). However, nerves do not seem to have been depicted in the paintings in question. Dr. Tulp's (1593-1674) dissection is confined to the arm, and apparently, some anatomical errors were committed in this painting by Rembrandt. It seems that the Dutch physicians of the XVIIth Century did not describe the nervous system. However, a quarter of a century later Peter the Great of Russia (1672-1725) during his 18 months (1697-1698) incognito trip to Western Europe visited, according to the Memoirs of his equerry, Vasily Volkov, anatomical collections in Zaandam, Holland and saw "the nerve on which the lung lives" and "the nerves which live in the brain … and … are like threads (see Tolstoi, 1959).

system were minimal; he was keenly interested in the structures of the brain and illustrated the dissection of the brain in his published work, yet, he had only a vague idea of the cortex and he considered the medulla to be the most important part of the brain. In the XVIIIth Century English and German physicians and anatomists, Winslow, Whytt, Meckel, Reil, Johnstone and Monroe (see Pick, 1970) expanded the knowledge of the autonomic ganglia ascertained the presence of sensory nerves in the ganglia, differentiated between the pre- and post- (efferent) ganglionic connections and corrected the significance of what was earlier called "intercostals", renaming them "nervi sympatici maximi" (Winslow, 1732). Johann Friedrich Meckel (1781-1833), a student of the great French comparative anatomist Georges Cuvier is the first investigator to give the name "ganglia" to the pertinent swellings, while Johann Christian Reil (1759-1813) referred to the system in question as "vegetative" (Reil, 1807; Meckel, 1821). Particularly Johnstone (1764), Winslow (1732) and Bichat (1802) stressed the complex morphology of the ganglia, the existence of their sympathetic outflow, and the involuntary and visceral nature of their influences as compared to the "relational life" guided by the brain. These investigators had a good understanding – before Claude Bernard, see below - of the homeostatic nature of the activity of the ganglia (Bichat, 1802), their control of visceral functions, and their role in linking emotions and certain visceral and what we would refer to as autonomic functions. In fact, Winslow and Johnstone referred to the ganglia as "little brains" [4].

The XIXth Century brought descriptions of unmyelinated postganglionc nerves (Ehrenberg, 1833; Remak, 1838), rami communicantes and pathways relaying ganglia to the spinal cord and to the effectors, as well as special ganglia such as otic and of nerve plexi (Meissner, 1857; Auerbach, 1864; see also Brazier, 1959; Pick, 1970; Ackernecht, 1974; and Karczmar, 1986). And the discovery of the smooth muscle in the iris, bladder, arterial wall, and gastrointestinal system (see Pick, 1970) provided bases for the understanding of involuntary (subsequently referred to as autonomic) functions. The evidence for actual activation by pertinent nerves of these and other structures included the brother Webers' (1845) confirmation of Willis' demonstration of the vagal effects on the heart; they used a galvanic pile as the source of the stimulus for the stimulus.

And then, a flurry of French, Swiss, German, Scot and mixed origin (in the case of Brown-Sequard) [5] investigators deepened the understanding of the function of the sympathetic, parasympathetic (without using this particular nomenclature), sensory and motor nerves. It should be stressed that all of them experimented on live animals [Magendie's work on animals evoked horror among some of his contemporaries (see Nordenskiold, 1936)]. Thus, the Edinburg-born Sir Charles Bell (1771-1842), a physician, founder of an Academy of Anatomy in London, and a military surgeon active as such at the battle of Waterloo (1814; see Nordenskiold, 1936) distinguished between dorsal and ventral nerve roots and their function (that Bell was discoverer of this dichotomy was disputed by Magendie). The French anatomist and academician Felix Vicq d'Azyr (1746-1794) developed the technique of coronal brain sections and he described for the first time the locus ceruleus, the locus niger, the claustrum and the including the mind – and the expressions of these functions on the surface of the skull. Marie F.-X. Bichat (1771-1802), a physician and later professor at Hotel-Dieu in Paris, described "le systeme de ganglions … controlant la vie organique". He maintained that life results from a combination of vitality and vital functions of various tissues of the body, thus rejecting reductionist concept of life and functions. Francois Magendie (1785-1855) was notable by his work concerning the sensory and motor nerves as well as circulatory processes and respiration, as well as by being the mentor of Marie-Jean Flourens (1794-1867) and Claude Bernard (1813-1878). Flourens continued working on nerve physiology, while

[4] Some forty years ago this author and his associates used the same term – "little brains" - for the ganglia, to denote the same notion of their complex morphology and function (Karczmar *et al.*, 1972), without quoting the originators of the notion and of the term; this was a case of innocence and ignorance, not of plagiarism. Our apologies, Professors Winslow and Johnstone!

[5] Charles-Edouard Brown -Sequard was born in the island of Mauritsius, and he resided in England, France and United Sates, to end his life as the successor of Claude Bernard as professor of Medicine Experimentelle at the College de France. His fame is derived not so much from the work described here, but from establishing the vital role of the adrenal glands and from his experiments with the testicles of guinea pigs and dogs as means for human rejuvenation … Thus, he established the role of substances referred to today as hormones.

Claude Bernard, deservedly famous for his work on the pancreas and glycogenic function of the liver, digestion, diabetes, homeostasis (or balance of the "milieu interieur"), the nature of motor function and the site of action of curare, obtained important data with respect to the effects of what we would call today sympathetic autonomics on blood circulation (Bernard, 1958). In the present context, his work with curare is of particular importance; indeed, his statements that "curare must act on terminal plates of motor nerves" and that "curare does nothing more than interrupt something motor which puts the nerve and the muscle into electrical relationship … required … for the movement …of the muscle"[6] suggests the presence of receptors and thus precedes the concepts of John Langley (see below, and Karczmar *et al.*, 2007)[6]. Charles-Edouard Brown- Sequard (1817-1894) who became Claude Bernard's successor as professor at College de France, had a most colorful past [4]. He was born was a son of an American sea captain and a young native of the island of Mauritius. Before settling in France, Brown-Sequard sojourned in the USA, Paris, Geneva and London; he studied in Paris (where he was befriended by Claude Bernard) and accepted medical positions in Virginia, at Harvard and in London; he gained a great reputation in London and dined there with the Prince of Wales, the future Edward the Seventh. Finally, he moved to Paris and he became Claude Bernard's successor as professor at the College de France. He continued the work of the French investigators on the sympathetic system, showing for example that, in animals, galvanic stimulation of sympathetic nerves cooled the skin and attenuated cutaneous blood flow (see Nordenskiold, 1936; Brazier, 1959; Pick, 1970; Rothschuh, 1973 and Karczmar, 1986). He also described the facilitation by the calabar bean extract of convulsions elicited by spinal cord lesions. And, Edme Felix Alfred Vulpian (1826-1887), a Paris physician and a collaborator of the great French founder of neurology, Jean-Martin Charcot (1825-1993) is even more pertinent to the subject of this article than the other French investigators referred to here: he worked with the extract of Pilocarpus jaborandi (Indian hemp; see below) and showed that the extract slowed the heart beat of animals; he believed that this effect occurs via the stimulation by Jaborandi of the endings of the vagus nerve article; he based this conclusion on his finding that curare antagonized this effect of the extract (more on this matter later, particularly in connection with the work of Langley).

On the German side, Johannes Peter Mueller (1801-1857) was a polymath distinguished for his many studies of marine animals, and of what he referred to as brain ganglion cells and their connections, as well for his philosophy of vitalism, as he wrote of "organic creative force". What is important in the context of this article is Mueller's continuation of the work of Magendie on spinal sensory and motor nerve roots and on the circulation, and his tutorship of important investigators such as Karl Friedrich Wilhelm Ludwig (1816-1895), Emil du Bois-Reymond (1818-1896), and E. F. W. Pfluger (1857) who established the role of what we would call today the parasympathetic system and of depressor nerve and reflex on the iris, salivation, vasodilation, gastrointestinal tract and genital organs (see, for example, Pfluger, 1857; Pfluger was also Charles Darwin's important correspondent). Their work was contemporary or followed closely by that of C.G. Ehrenberg, R. Remak. G. Meissner Leopold Auerbach, B. Luchsinger, R. Goltz and Moriz Schiff (1817-1894) [7]; they studied rami communicantes and pathways relating autonomic ganglia to the spinal cord and to the effectors, special ganglia such as otic, the plexi such as enteric myenteric plexus, and pilomotor activity, cardio-accelerator function of sympathetic nerves, and nerve control of digestion (Rothschuh, 1973; Brooks & Seller, 1981; Karczmar, 1986).

Du Bois-Reymond's significance in the context of this article is that he proposed as an option that the muscle effect of motor nerves could be due to the liberation by the motor of nerves of a chemical substance (see below; du Bois-Reymond, 1843). An important additional step made by du Bois-Reymond was his discovery of the latency between the electric current carried by the motor nerve and its invasion of the muscle; this discovery should be linked with the demonstration in 1862 of histological discontinuity between nerve and muscle by Wilhelm Kuehne (in 1877 Langley worked in Kuhne's laboratory). This

[6] In 1856, the German investigator Arnold von Koelliker described at a session of the French Academy of Sciences the "extinguishing" effect of curare at the motor verves; he was apparently unaware of the earlier, more extensive and more precise results obtained by Claude Bernard, so Bernard rushed to present, again, his results at the next session of the Academy (see Holmstedt and Liljenstrand, 1963, and Karczmar er al., 2007).

[7] Moriz Schiff was a member of a Jewish Frankfort-on-Main family prominent since early XIVth Century that included scientists, doctors, bankers and rabbis.

discontinuity is analogous to that subsequently demonstrated for central as well ganglionic synapses (the term coined by Sir Charles Sherrington; see below) by the Czech Johannes Purkinye (1787-1869), the Swiss Rudolph Albert von Koelliker (1817-1905), the Italian Camillo Golgi (1843-1926) and the great Santiago Ramon y Cajal (1881, 1911) who shared the Nobel Prize with Purkinje and Golgi. Their discoveries established the principle of neuronal structure of the brain and the ganglia and they were necessary for establishement of the chemical nature of transmission.

Additional advances in the knowledge of the brain were made in the XIXth Century by the German medical men and physiologists, particularly the students of Johannes Mueller and Karl Ludwig, Karl Wernicke (1848-1904), Gustav Fritsch (1838-1907), Korbinian Brodmann (1868-1918) and Eduard Hitzig (18138-1927). Wernicke and his predecessor the French physician Pierre Paul Broca (1824-1880) established the language and the speech center (Broca's area) and further localization of functions and behaviors in the brain was provided by Fritsch, Hitzig and Brodmann, the latter particularly with regard to the cortical localization of functions. And Jean-Martin Charcot (1825-1893), the French neurologist who served for 33 years as the Chief Medical Officer of the Salpetriere Hospital of Paris and who is famous for the quality of his pupils who included Sigmund Freud, Joseph Babinski, William James and Georges Gilles de la Tourette, established brain links of a number of neurological disorders, including (with James Parkinson) Parkinson-Charcot disease, multiple sclerosis, hypnosis and hysteria. It must be added and emphasized that, while among the neuroscientists discussed above Claude Bernard, Du Bois-Reymond and several others touched upon the matter of receptors and alluded, tentatively, to the option of chemical or transmitter-mediated neuronal and ganglionic signaling, the work did not include any real forays into the matter of transmitters and the nicotinic and muscarinic transmission modes!

This then leads to the epochal and definitive establishment of the autonomic nervous system, by the three Cambridge, England investigators, Walter Holbrook Gaskell (1847-1914), John Newport Langley (1852-1925) and William Lee Dickinson (1863-1904), and to the mapping of brain parts and their behavioral and functional significance by the American Karl Lashley (1890-1958), the Canadian-born American Wilder Penfield (1891-1976), the Philadelphian Horace Winchell Magoun (1907-1991), and others.

These neurologists, neuroscientists and neurophysiologists, some of their immediate predecessors, their contemporaries and followers could not elucidate the anatomy and function of what became known as autonomic nervous system without using the extracts containing active alkaloids and, later, their purified and/or synthetic principles, atropine, pilocarpine, physostigmine, d-tubocurarine, nicotine and, importantly in the context of this article, muscarine; it would be then inappropriate to dissociate the story of these alkaloids from that of the autonomic and central nervous system, or to neglect this story. Accordingly, a short description of the stories of physostigmine, anticholinesterases (antiChEs) other than physostigmine, particularly the non-alkaloidal, synthetic organophosphorus (OP) antiChEs will be forthcoming.

Indeed, Sir John Henry Gaddum (1954), the great British explorer, on his own as well as with Sir Henry Dale, Ulf von Euler and others, of the skeletal neuromyal junction and autonomic and central nervous systems, as well as David Nachmansohn (1899-1983), the Russian-born American investigator (active in Germany and France before settling in New York), the discoverer of choline acetylase (cholineacetyltransferase; CAT), and the pioneering student of cholinesterases and the cholinergic system, both opined that antiChEs were and are crucial in the discoveries and studies of the autonomic and central cholinergic systems. Their statements concerns also the use of other than physostigmine or carbamate antiChEs and of OP drugs, and it could be as justifiably quoted with regard to the studies of atropine, nicotine, pilocarpine and d-tubocurarine.

2. The Early Story of Pharmacologically Active Alkaloids and of OP AntiChEs

Atropine is present in many Solanaceae plants, such as deadly nightshade (henbane or Atropa belladonna), jimson weed (Datura strammonium), mandrake (Mandragora officinarum) and Hyoscyamus niger. Presumably, their extracts were known to pharaonic Egyptians (Karczmar, 2007). Historically,

the first Solanaceae plant that was shown to have medicinal properties was mandrake, described by Theophrastus (371-287 B. C.) as useful for the treatment of gout and sleeplessness. Many centuries later another Greek Pedanius Dioscorides (ca. 40 - 90 A. D.) used mandrake for sleeplessness and in presurgical anesthesia. In Roman days, Sollanaceae extracts were employed as poisons, and Cleopatra allegedly used them to dilate and beautify her eyes, a custom followed later by the ladies of Renaissance and of Paris, hence the name Belladonna. And the Roman historian Lucan describes how some of the Roman soldiers marching through Northern Africa during the civic war between Caesar and Pompey were poisoned by ingesting mandrake or some other Solanaceae plants. Then, the Baroque scholar Antoni Strock stated that "many" or "all" authors wrote: "Strammonium disrupts the mind, induces madness, erases ideas and memory, brings about convulsions" (Storck 1757; see also Wassen, 1965). The usefulness of the mydriatic effect of Atropa in ophthalmology was proposed in the eighteen fifties by the German chemist Friedrich Ferdinand Runge (1795-1867); further employment of Atropa extracts and synthetic atropine in the studies of the autonomic nervous system are describe in the next Section of this article. The German pharmacist Mein isolated, purified and crystallized atropine, and the German chemist and Nobelist, Richard Willstaetter (1872-1944) established in 1901 atropine's structure and synthesized it.

Several species of Rutaceae, such as Pilocarpus jaborandi and Pilocarpus microphyllus (Indian Hemp, Jaborandi) contain pilocarpine. These shrubs are present in South America, particularly in Brazil, and to a lesser extent in Central America. Gabriel de Souza was the first to observe in 1570 the Brazilian Guarani Indians using the plant to treat mouth ulcers. In 1663 two Dutch West Indian Company scientists observed the Brazilian Indians using Jaborandi for many purposes, including the treatment of colds and gonorrhea and as antidote against a number of plant toxins; this latter employment may have been based of Jaborandi's diaphoretic, urinary, lachrymatory and salivatory effect (Holmstedt *et al.*, 1979); in fact, Bo Holmstedt travelled in the nineteen seventies to Amazonas, stayed with the Amazons Indians and observed their multiple use of Jaborandi (Karczmar, 2007). In modern days, this effect was replicated in animals and referred to as SLUD (salivation, lachrimation, urination, defecation). In 1873, Symphronio Coutinho, a Brazilian physician travelled with the leaves of Jaborandi to Paris, where the French physicians used the leaves in clinical research and confirmed that "…Jaborandi …increases enormously the perspiration, and saliva, and, in a much less degree, the secretion from the mucous membranes of the nose, the bronchial tubes, and the stomach and intestines". In 1875, both M. Hardy and A. Gerrard purified and indentified pilocarpine as the active ingredient of Jaborandi; and Hardy with T. Kamel synthesized pilocarpine in 1887 (Wollensak & Kewitz, 1976; there is some controversy as to their synthesis). This then led almost immediately to its employment in ophthalmology (as a miotic and antagonist of atropine; Holmstedt & Liljenstrand, 1963; Holmstedt *et al.*, 1979), and further autonomic studies of Jaborandi or synthetic pilocarpine will be described below.

The alkaloid nicotine is found in the tobacco plant of the genus Nicotiana tabacum (a part of the nightshade family); there are many species within this genus. The Spanish term "tabaco" may be derived from the term used by Carribean tribe of Arakawas; however, the name come also from the Arabic term "tabbaq", a plant used medicinally for its psychoactivity by the Arabs since the IXth Century. The XVth and XVIth Centuries sailors and explorers became cognizant of the use of tobacco in the West Indies and Central America and brought the plant to Europe. For example, Christopher Columbus (1451-1506) described the use of tobacco among native Indians (see also the letter of D. A. Chanca, Columbus' medical officer on Columbus' second voyage to America, published in Spanish in Seville around 1550 and available today in the Italian translation, 1989). And the French explorer Jaques Cartier (1491-1667) smoked tobacco with the native Americans in the course of his three voyages to New Fundland and to today's Canada. Of course, for centuries before the voyages of these explorers tobacco was smoked by the natives of the Americas, and as already indicated, it may have been also used by the Arabs. Among the native Americans its use was confined to religious and social ceremonies (as an entheogen). However, in Europe tobacco became desirable as a cure for mental afflictions and all kinds of disease. In England, Sir Walter Raleigh smoked tobacco and he induced Queen Elizabeth the First to use it as well. In Paris, Jean Nicot de Villemain who was sent tobacco and tobacco seeds propagated the medicinal use of tobacco in France and introduced it in 1559 to the Queen Marie de Medicis; actually,

the plant nicotiana was named after him. Rapidly, its use, whether recreational or medicinal spread all over Europe, including Russia: Tsar Peter the Great, in his effort to westernize Russia, introduced this western delight into Russia, and in fact, forced his boyars to use tobacco.

In 1828, pure nicotine was isolated from the tobacco plant in Germany by Posselt and Reichmann; its chemical empirical formula was established by Melsens (1844) and G. Pinner described its structure in 1893, while its synthesis was accomplished by Pictet and Crepieux in 1904. The pertinent experimental work with nicotine extracts (and, much later, with synthetic nicotine) will be described below.

Curare was prepared by the Amazonian Indians, as also by other South American tribes from bark and roots of several species of Strychnos and Menispermacea family that includes the Chondodedron tomentosum. It was used in arrows, mixed with other substances and in blow-guns, referred to by the Spaniard Cieza De Leon (1553) as "sarabatana". Actually, the references by many Spanish conquistadores and Spanish and Italian chroniclers (such as Pietro Martire D'Anaghera in his letter of 1516 to the Pope Leon X, Gonzalo Fernandez Oviedo y Valdez, the companion of Gonzalo Pizarro and Francisco Orallano) to sarabatana or arrow poisons, may have dealt not only with curare but also with other poisons (Vellard, 1959). In fact, the earliest description of the effect of the poisons used in sarabanes that indeed are curare-like in our modern understanding date only from much later than the documents listed above; in this case, Lucas Fernandez de Piedrahita quotes an earlier explorer, Alfonso Perez de Tolosa who found that the poison produces a "lethargy" or immobility (see Ribeiro & De Melo Carvalho, 1959). And, the term "curare" seems not to have appeared till Walter Raleigh used the term spelling it "Ourari" in 1596 (see the posthumous edition of his work in 1848); there are many additional spellings of this word. At any rate, it appears that the term originated in Guyana where it referred to a species of a Strychnos family. And the great explorer and ethnographer Alexander von Humboldt described in 1800 how the toxin was prepared from Strychnos plants by river Orinoco natives. Yet, the isolation of the pure active alkaloid of Strychnos had to wait till the researches in the nineteen thirties of H. Wieland, O. Wintersteiner and several other German and Swiss chemists (see Bovet *et al.*, 1959); in 1935 Harold King, working in the laboratory of Henry Dale, isolated pure d-tubocurarine and proposed a chemical structure for this compound, although the definitive structure was not established till 1970. Finally, a number of laboratories in Switzerland, Germany, Italy and USA contributed to the methodology of synthesizing d-tubocurarine (see Fusco *et al.*, 1949 and Bovet, 1957). Experimentation with curare pertinent to this chapter, besides the Claude Bernard's famous work that was already mentioned will be discussed subsequently.

The Ordeal Bean that contains the active alkaloid, Physostigma venenosum was known since the eighteen fifties to the British explorers of and Scottish Edinburgh missionairies in, the Old Calabar, a province of Nigeria; it was brought from Nigeria to Scotland by Daniell and it was identified as Physostigma in 1861 by the Scot John Hutton Balfour (see Bo Holmstedt's grasping review, 1972; see also Simmons, 1956, Wassen & Holmstedt, 1963 and Karczmar, 1970, 1986 and 2007). This scientific name is derived from the beak-like appendage at the end of the stigma, in the center of the Physostigma flower; this appendage is solid, but it was thought to be hollow, hence the Greek name for bladder, "fisa" being used in the term Physostigma. According to the Scot missionairies, in its use as a "truth bean" the Elfiks of Old Calabar made the suspected criminal swallow 6 beans, macerated in water. Most likely, an innocent subjected to the trial would swallow the beans without hesitation, the beans would form a bolus, eliciting a life- saving vomiting. However, the bean was also used in Africa including Egypt and other tropical lands such as South China medicinally as well as an antidote to curare and other poisons (Levey, 1966; Hanin *et al.*, 1991; Karczmar, 2007). The Scottish missionairies published their ethnographis and botanical findings in the Scotland's Missionary Record and provided a link to the physicians and pharmacologists of Edinburgh who initiated the scientific phase of the Physostigma research (see Gaddum, 1962 and Holmstedt, 1972). This phase included Robert Christison's, a Professor of Materia Medica and Clinical Medicine at the University of Edinburgh self-experimentation with the bean (1855). He recorded "a morphia-like" soporific action, "giddiness … torpidity …very feeble … irregular … pulse and the action of the heart … muscle cramps". Using "shaving water" that he just used as an emetic the energetic 78 years old man barely recovered (Holmstedt, 1972). And Christison, Thomas Fraser, Christison's successor to the Edinburgh chair, and Alexander Robertson provided additional data as to the actions of the bean, including respiratory depression, skeletal muscle effects

terminating in paralysis, miosis and intestinal hyperactivity (they advocated using the bean in the treatment of cholera). Indeed, Robertson (1863) discovered the use of the bean as extract in ophthalmology, as the antagonist of atropinic mydriasis. And Fraser described the use of the bean extract as an antagonist of strychnine-induced seizures [8].

The first recorded occurrence of physostigmine – or the Calabar bean - toxicity dates from these Edinburgh days. A shipment of Calabar bean was brought to Liverpool in 1864 for its medicinal and pharmacological use in Edinburgh, and 42 Liverpool children consumed the bean, present in the " …sweepings" from the ship in question. They showed signs of toxicity which a London physician, John Cameron (1864) treated with "emetics and a plentiful supply of warm water and brandy". One child died, and this death could be perhaps avoided if Cameron was aware of the Edinburgh's postulated atropine-bean antagonism…

The Edinburgh studies were followed by extensive French, German, English and American investigations which have a bearing on both autonomic and central muscarinic transmission; they will be reviewed subsequently. The use of physostigmine in this work was facilitated by its isolation, purification and synthesis. The German chemists Jobst and Hesse (1864) were the first to isolate the principle of the Ordeal Bean and Physostigma venenosum which they called "physostigmine"; a year later Vee and Leeven (1865) in France crystallized the active principle and called it "eserine", from the Calabar bean native name "essere" (see Karczmar, 2007). Polonowski and Nitzburg (1915), Stedman and Barger (1925) and Petcher and Pauling (1973) [9] defined the structure of physostigmine; however, Percy Lavon Julian (Julian & Pike, 1935) [10] managed to synthesize physostigmine before its structure was definitely clarified. It must be noticed that, in spite of all the many years of studies of the actions of the bean, including its toxic effects and of the knowledge of its structure, the antiChE action of the bean or of physostigmine did not become known till 1930 when Kurt Matthies and Otto Loewi himself (Engelhardt & Loewi, 1930) demonstrated that physostigmine inhibits blood hydrolysis of acetylcholine (ACh).

This synthesis and the knowledge of the indole-carbamate structure of physostigmine prompted the synthesis of many derivatives of physostigmine, such as clinically useful quatenary prostigmine (neostigmine); several alkaloids such as huperzine which is used in the treatment of Alzheimer disease (Hanin *et al.*, 1991) share the reversible mode of antiChE action of physostigmine, but are not indole carbamates (see Long, 1963; Karczmar, 2007).

The OP agents were first synthesized in eighteen twenties by Jean-Louis Lassaigne, a Professor at Alfort's (in France) Ecole Royale Veterinaire; as a well-known toxicologist he may have studied their toxic effects (see Fest & Schmidt, 1970; Eto, 1974; Chambers, 1992; Karczmar, 2007). And, sometimes in the middle of the XIXth Century, a certain M. Moschnine, working in the laboratories of Charles-Adolphe Wurtz, a great French chemist (born in Germany), synthesized a prototype of war gases, tetra ethyl pyrophosphate (TEPP). Moschnine, probably a Russian by origin was not further identified, and the credit for his discvovery was given to Paul de Clermont who also synthesized TEPP (Clermont, 1854). Actually, de Clermont tasted TEPP by swallowing some amount of it (TEPP is a liquid). As suggested by Bo Holmstedt (1963) the fact that de Clermont did not succumb to TEPP, a very potent poison, obscures the picture of OP agents… In fact, de Clermont lived to the ripe age of 91 years. Parenthetically, many investigators, beginning with the Swede P. Nylen and including this author worked with TEPP for many years without suffering any ill effects, and actually Nylen (1930) did not even

[8] Charles Brown-Sequard, already referred-to (and also mentioned in Footnote 4) found, somewhat contradictorily, that seizures induced by certain types of spinal cord lesions, are facilitated by ordeal bean extracts. These early findings constitute just the beginning of many controversial studies of the effect of antiChEs on seizures (see Karczmar, 1974).

[9] Peter Pauling was the son of Linus Pauling, a double Nobel Prize winner. It is not easy to be a son of a genius, and Peter was not satisfied with being a distinguished and brilliant stereochemist; his short life was not a happy one…

[10] The late Percy Julian (1899-1975), the author's friend and neighbor in Oak Park, Illinois (also the birth place of Ernest Hemingway) was famous not so much for his synthesis of physostigmine but for that of corticosteroids and sex steroids. These syntheses were patented and Julian became a rich man.

realize that TEPP is toxic… Another early synthetizer of OP drugs was von Hoffman (1873) who worked both in England and Germany.

The Arbusovs, a duo of father and son developed, at the beginning of XIXth Century a number of methods for synthesizing the OP agents (see Holmstedt, 1963; Karczmar, 2007). But, the main force behind the intense synthesis and pharmacological development of OP drugs is due to German investigators working either in the German Universities or for the IG Farbenindustire such as C. A. A. Michaelis, Willy Lange, Gerda von Krueger and Gerhard Schraeder (Holmstedt, 1963 and 2000); in the course of synthetic work they recognized in the nineteen thirties their toxicity (Lange, personal letter to Bo Holmstedt, April 7, 1952; see Holmstedt, 2000) as well as their potential as insecticides and pesticides. The toxicity of OP drugs became known to the German Government which then initiated an intense development, led by Wolfgang Wirth, of OP war gases. And, as soon as the UK and USA government became aware – both via private communications and Secret Service activities – of this effort, the same endeavor took place in these two countries. The UK effort, organized by the Ministry of Supply and led by Lord Adrian was centered at Porton Downs, while the USA team worked mainly at Edgewood Arsenal, Maryland, and Walter Reed Hospital, Washington, D. C. (see Holmstedt, 1963 and 2000, Karczmar, 1970 and 2007; Usdin, 1970). This particular story as well as that concerning the development of OP agents as insecticides is outside the scope of this article; yet, certain aspects of this development, including the Nazi past of Wirth and his role in experimentation on humans – in concentration camps and elsewhere – with OP drugs merits attention (see Loeffelholz, 2000 and Karczmar, 2007).

What is pertinent here is that H. Gremels and Eberhard Gross, members of Wirth's team, recognized in the nineteen forties the antiChE properties of the OP agent, Tabun (also a war gas) and TEPP, and similar findings with regard to another OP agent were published in open literature by Swiss investigators (see Karczmar, 1970). Furthermore, another member of the German team, L. Lendle, found that Tabun potentiates in animals the vasodepressor effect of ACh, and the parasympathetic effects of the OP drugs became also known to the German scientists (see Homstedt, 2000). Parallel observations were made at the same time by the Porton Downs team, which included Bernard Kilby and William (subsequently Sir William) Feldberg; Macworth and Webb (1948), members of this team, were the first to recognize the irreversible nature of the cholinesterase inhibition by these agents, in contradistinction to the reversible nature of the inhibition exerted, as already mentioned, by carbamates and related antiChEs. And di-isopropyl phosphofluoridate (DFP), a compound used later extensively in the studies of the autonomic and the cholinergic central nervous system was synthesized by McCombie and Saunders, the members of the UK team. Similar work was carried out by American scientists working at or with, several centers, including Edgewood, Maryland and Reed Hospital, Washington, D. C. These scientists included George Koelle, Alfred Gilman, Amadeo Marrazzi, Bernie McNamara, Stephen Krop, Theodore Koppanyi, Henry Wills and many others (see Koelle & Gilman, 1949; Saunders, 1957; O'Brien, 1960; Heath, 1961). These investigators described, besides the toxic effects of OP insecticides as well as war gases, their parasympathetic, sympathetic (ganglionic) and central effects. These and subsequent investigations will be described below in this article.

The last substance to be described here is the alkaloid muscarine which is, as it will be seen, most pertinent to this story. It is derived from the brilliant orange-cap mushroom Amanita muscaria; it is present also in several species of the genus Inocybe (Wilkinson, 1961). The name of the species – muscaria – refers to the early use of the mushroom as a fly poison; hence the common name "fly agaric". This use of the ingredients of the extracts of A. muscaria was first recorded by Albertus Magnus sometimes around 1250. Actually, the insecticidal action of Amanita is not due to muscarine but to another constituent of this mushroom, the ibotenic acid. Linnaeus classified the mushroom in question as Agaricus muscarius, but Lamarck gave it the name Amanita muscaria, which is the name that stuck (Wikipedia, June 2009). While toxic, the fly agaric rarely causes death, while the ingestion of the related species, Amanita phalloides (death cup) is frequently fatal; however, the active agents of A. muscaria are peptides, not related to muscarine (Wikipedia, June 2009). The French investigators of the late XVIIIth and early XIXth Century such as Louis Nicolas Letellier (1763-1829) were the first to attempt to identify the active ingredients of the fly agaric; actual isolation and identification of muscarine had to wait till the partial isolation of muscarine by Oswald Schmiedeberg (Schmiedeberg & Koppe,

1869), considered by many as the Father of pharmacology because of his introduction of systematic methodology for studies of pharmacological effects of many substances besides muscarine (see Holmstedt & Liljestrand, 1963). And Rudolf Harnack (1875) was the first to propose a chemical structure for muscarine. The attempts to isolate muscarine in its pure form, to define its structure and to synthesize it continued through the next eighty years (Wilkinson, 1961). The Swiss chemist C. H. Eugster, working with the famous cholinergiker Pater Waser were the first to obtain pure crystalline muscarine chloride (Eugster & Waser, 1954); Eugster, Koegl and Kuehl (see Wilkinson, 1961) proposed a definitive structure for muscarine and they also managed to synthesize it, while Laszlo Gyermek with Klaus Unna (1958) and Peter Waser established that, thus identified muscarine exhibits specific actions at parasympathetic effector sites.

Actually the central effects of poisoning with A. muscaria are not due to muscarine, as the latter penetrates but poorly the CNS, although poisoning with the fly causes in man toxic autonomic actions, presumably due to its muscarine content, such as low blood pressure, sweating, intestinal reactions and salivation, and SLUD syndrome (salivation, lachrymation, urination and defacation) in animals; the central hallucinatory and toxic actions of the fly are due to other than muscarine constituents of the fly agaric, muscimol and ibotenic acid, as shown by many researchers in England, Switzerland and Japan (see, for example, Bowden & Drysdale, 1965 and Eugster *et al.*, 1965; see also Wikipedia, June 2009). Actually, to demonstrate the effects of muscarine on the central cholinoceptive neurons, microelectrode and micropipette methods of the application of muscarine are needed (see below). And A. muscaria extracts and, subsequently, muscarine were employed extensively in the analysis of the autonomic nervous system, including the vagus nerve, and this work led to coining the term "muscarinic receptors" (MRs; see below).

On the other hand, A. muscaria was used for millennia for their psychoactive properties by whole populations, and by the shamans and sorcerers of Asia (including Siberia, Afghanistan and India), subarctic Indian tribes and, possibly, in what is today Finland (see Wikipedia, June 2009). And the Swede Samuel Oedman (1784) suggested that A. muscaria was used by the medieval Vikings to produce their battle (berserk) rage. In fact, Richard Gordon Wasson (1898-1988), a banker and mycologist travelled to India and elsewhere to trace this use, and he maintained that A. muscaria was a constituent of the Rig Veda's soma; this notion is controversial, and the plant producing soma was, most likely, ephedra (Wasson, 1968; Wasson & Ingalls, 1971) [11].

3. From Gaskell's and Langley's Definition of Autonomic Nervous System to Loewi's Demonstration of Peripheral Chemical, Cholinergic Transmission

The studies of William Gaskell and John Langley with William Dickinson, as well as those of Oswald Schmiedeberg, Richard Koppe, Reid Hunt, Walter Ernest Dixon and Sir Henry Hallett Dale provide an excellent example of correlation between morphology of the autonomic nervous system and its function. Langley's senior colleague, Gaskell (1886, 1916) provided the anatomical and functional bases for the autonomic system as we know it today: following the work of Robert Remak, already quoted, Gaskell traced the anterior and posterior roots of cranial nerves of the "vertebral or lateral ganglionic chain" and described the three separate outflows of the fibers, today referred-to as preganglionic: bulbar, thoraco-lumbar and sacral; to him, these outflows constituted the involuntary nervous system. He also described prevertebral or collateral ganglia, and he clarified the role of posterior, sensory spinal roots as distinct from the splanchnic, preganglionic roots. He studied the control of the vagosympathetic and cranial nerves (Gaskell, 1886) with regard to cardiac, papillary, salivary and lachrymal activities. He posited the presence of a reflex arc consisting of an afferent nerve terminating

[11] Soma was the term used by Aldous Huxley in his famous book, "The Brave New World"; according to Huxley, in his future new world soma was used as a non-toxic tranquilizer and psychic stimulant ("better a gram than a damn"); the term "soma" was also used in a similar sense by William S. Burroughs in his book, "Naked Lunch". In fact, "soma" is the brand name of the minor tranquilizer Carisoprodol.

in the lateral horn or vagal nuclei and a connector nerve uniting the lateral horn or the vagal nuclei with the sympathetic or vagal ganglion cells. Of particular importance is Gaskell's statement that "every … involuntary … tissue is innervated by two sets of nerve fibers of opposite characters". Finally, it was prescient on Gaskell's part to suggest that the central nervous system is regulated by inhibitions analogous to those that he described for his involuntary system! It should be added that Gaskell liked to use young crocodiles for his experiments, particularly on the heart and the vagal and sympathetic regulation of its beat…

John Langley, the Chair of Physiology at Cambridge continued and brought to new hights his Cambridge predecessor Gaskell's studies. It should be added that while Gaskell used nicotine and pilocarpine as tools rather sporadically, Langley did so quite consistently. Thus, with his associate William Lee Dickinson of Caius College, Cambridge, Langley mapped the autonomic nervous system – the term that Langley coined, as well as the terms of parasympathetic ouflow – that regulates "the involuntary" effectors; he relied on nicotine's capacity to initially stimulate and subsequently block temporarily the ganglia; for example, "after a moderate dose of nicotine, stimulation of the sympathetic nerve causes no dilatation of the eye", and Langley proved that this block is due to nicotine's action on the ganglia by applying nicotine to the ganglion itself (Langley & Dickinson, 1889; see also Fletcher, 1926; Holmstedt & Liljenstrand, 1963; Karczmar, 1986; and Maehle, 2004). In this way, Langley disproved the notion of his contemporary's Hirschmann (1863) that nicotine paralyzes the nerve endings of the sympathetic outflow, the related view of another contemporary, the German investigator Michael Rossbach, as well as the earlier proposal of Vulpian (see above) that Jaborandi stimulates the inhibitory vagal endings; indeed, Langley stated that jaborandi extract – or pilocarpine - "produces this slowing by acting on something else than the inhibitory nerve-fibers going to the heart" (Langley, 1875b; see also above), basing this claim, at least in part, on his findings that '"atropia" antagonizes this effect and that this antagonism "depends on the relative amounts of jaborandi and atropia present" (Langley, 1875a and b); he demonstrated these effects with regard to the heart rate of several vertebrate species. He also used the jaborandi extract and the Calabar bean extract – or physostigmine – in his studies of salivary secretion, the submaxillary gland and vagal cardiac effects, and he showed the antagonism between these alkaloids and "atropia", as well as the capacity of atropine to block the effects of the electrical stimulation of chorda tympani and the vagus; his work on the frog's heart expanded on that of Sir Michael Foster, his predecessor as Professor of Physiology at Cambridge. Interestingly, a few years later the Zurich physiologist Balthasar Luchsinger showed that similar antagonism exists with regard to cat's sweat (Luchsinger, 1877). These and other studies led to Langley's and Dickinson's demonstration that Gaskell's cranial and sacral outflows constitute the parasympathetic nervous system – another term invented by Langley. He also showed that these two outflows respond to pilocarpine, while adrenaline is active at the thoraco-lumbar system. And Langley thoroughly investigated the distribution of the pre- and post-ganglionic nerve fibers. Also, Langley recognized that the plexi of Auerbach and Meissner constitute a system which is independent of the autonomic "enteric system", and which has "no significant connections with the autonomic outflow from the spinal cord" [Ramon y Cajal's subsequent contribution (1892) to this subject is important].

As important as these results and concepts may be, Langley's formulation of the receptor concept may overshadow his other accomplishments. A number of steps were taken by Langley (and his student Elliott) to arrive at this concept which replaced his earlier idea of the action of jaborandi and atropia at nerve endings. First, he described his and Luchsinger's findings, stressing that the antagonism between two substances such as atropine and pilocarpine depends on "the mass of each … substance … substance present" (Langley, 1880). Then, he stressed that pilocarpine could produce salivation even when the effect of the stimulation of the chorda tympani was blocked by atropine. Finally, he demonstrated that nicotine applied to the superior cervical ganglion and the skeletal muscle still induced its typical, first excitatory and the blocking actions on the effectors even after the preganglionic and motor nerves were cut and allowed to degenerate – hence, nicotine did not act via the preganglionic nerve endings. This work was criticized by two British investigators, Thomas Gregor Brodie (1866-1916) and Walter Ernest Dixon (1871-1931), as they felt that the time allotted by Langley for the nerve degeneration was insufficient; similar arguments against Langley's concepts were raised by the German investigators,

Hermann Fuhner (1871-1944) and Rudolf Magnus (1873-1927); to refute their opinions, Langley repeated his work allowing fourteen and a half month for the preganglionic nerve degeneration (Langley, 1905). And another Cambridge man, Walter Dixon, the lecturer in pharmacology at Cambridge initially preferred to think of receptors as underlying internal secretion and hormonal actions (Dixon, 1907) – as will be seen later, Dixon changed later his mind. Finally, Walther Straub (1874-1944) developed the "Potentialgifttheorie"; according to this theory the action of "poisons" such as muscarine was due to the cell saturation with the poison and the development of a gradient of the poison between the cell membrane and its inside. As pointed out by Langley (1907), Straub's theory applied to the poisonous effect of drugs such as muscarine and jaborandi, rather than to their stimulating effect (see also Maehle, 2004).

And, Langley realized that his findings, were in agreement with the concepts of von Koelliker and Ramon y Cajal (see above) as to the neuronal, discontinual character of the brain and as to the discontinuity between nerve endings and nerve cells, as well as nerve endings and effectors such as the skeletal muscle. He was also impressed by his English contemporaries, George Oliver (1841-1915) and Edward Albert Schafer (1850-1935) and the German investigators Max Lewandowsky (1876-1918) and Neinrich Boruttau (1869-1923) that demonstrated that the extract of the suprarenal gland – that contains adrenaline (epinephrine) – acts on the smooth muscle of the blood vessels and of the eye, as well as on the striatal muscle (Pick, 1970; Maehle, 2004).

Actually, Langley's own student, Thomas Renton Elliott (1877 – 1961) expanded the work of these four investigators as he proved that the effect of the suprarenal extract, and also adrenaline (isolated from the suprarenal gland by Jokichi Takamine; see Voegtlin, 1939) simulates the effect of electrical excitation of the pertinent sympathetic nerves. In fact, Elliott proposed at the time that adrenaline is "a chemical stimulant liberated when the … nervous … impulse arrives at the periphery" and acts not so much at the effector, such as the muscle, but at a "substance" which constituted the "myoneural junction", a clear statement of a transmitter mechanism (Elliott, 1904; see also Holmstedt & Liljenstrand, 1963 and Maehle, 2004). And Langley added some experiments with adrenaline of his own to help Elliott winning the argument from Dixon and Brodie (see above).

So, by 1905 Langley was ready with stating his receptor hypothesis (1905 and 1906), as he described the "receptive substances" that are separate from the cells' "chief substance which is concerned with the chief function of the cell such as contraction and secretion"; … the receptive substances … are acted upon by chemical bodies and in certain cases by nervous stimuli. The receptive substance affects or is capable of affecting the metabolism of the chief substance". It must be added that, as stressed by Maehle (o.c.), Langley located his receptive substance *in* the cell, rather as on the cell; thus, his receptor concept differed from the modern concept.

Today, we refer to Langley's "receptor substance" as the receptor; It is considered today that the definitive formulation of the receptor concept is due to the efforts of both Langley and the great German bacteriologist and chemotherapeutist, Paul Ehrlich (1854-1915; see Wasserman, 1915 and Holmstedt & Liljenstrand, 1963), although Langley's formulation of his "receptive substance" preceded Ehrlich's selectivity hypothesis; Ehrlich did use the term "receptor", and he coined the aphorism, "Corpora non agunt nixi fixata". Ehrlich studied the selectivity of chemotherapeutic actions using the term "binding" and he developed the "Seitenketten-Theorie" to explain the mechanisms of this binding. Interestingly, he quoted Oswald Schmiedeberg (1838-1921) with regard to Schmiedeberg's opinion that pharmacological agents exert a "chemical" action upon the organism [12]. Langley was not only aware of Ehrlich's work, but quoted him and applied Ehrlich's side chain theory to his "receptive substance" concept, as he described these substances as "atom-groups of the protoplasm" that could be

[12] Oswald Schmiedeberg, a native of Dorpat and a student of Rudolf Buchheim (1820-1879, one of the early pharmacologists and students of Materia Medica), was first the Professor of Pharmacology at Dorpat and then the founder and Director of Strassburg's Institute of Pharmacology. He is considered to be the father of modern pharmacology and his students became professors of pharmacology all over Europe; in fact, three early USA professors of pharmacology, John Jacob Abel, the first USA Professor of pharmacology at Ann Arbor, Michigan and then at Johns Hopkins, Baltimore, Arthur Robertson Cushny who succeeded Abel at Ann Arbor, and G. M. Wallace, professor of Pharmacology in New York, were Schmiedeberg's students.

"fundamental" and cause cell death when bound by appropriate chemicals, or "receptive" and altering cell function when affected by such agents as alkaloids (Limbird, 2004; Maehle, 2004).

It is piquant that the great Sir Henry Dale (with George Barger; Barger & Dale, 1910) argued with Langley's receptor thesis with regard to their finding that many ("sympathomimetic) amines mimicked the effects of sympathetic nerve stimulation without having any common structural components. Ultimately, Dale changed his mind, and subsequent work of Langley's pupil, Archibald Vivian Hill (1866-1977) and Alfred Joseph Clark (1885-1941), a professor of pharmacology at Edinburgh, who both applied a mathematical analysis to Langley's work with nicotine and atropine, as well as with ACh and sympathomimetics provided additional, strong basis for Langley' s redeptor concept; they all used the term "receptor" rather than the more complex term "receptive substance" (Holmstedt & Liljenstrand, 1963; Karczmar, 1986; Maehle, 2004).

The neuronal and neuromyal junction discontinuity theory and the concept of receptors begged for the postulaton and discovery of transmitters, since how would nerves communicate with the receptors and cells without a chemical messenger? Yet, the task of demonstrating chemical transmission on whether autonomic, skeletal and smooth muscle, and central sites proved arduous. As explained above, Galen, Vesalius and several Renaissance and post-Renaissance investigators spoke of "spirits" as messengers, via the nerves, of effector function, while Francis Glisson (1597- 1677), Alessandro Volta (1745-1827) and Luigi Galvani (1706-1777) proposed that the communication in question is due to an electric (or galvanic) force (see Brazier, 1959 and Castiglioni, 1947). Finally, some three hundred years after Vesalius, du Bois-Reymond and Claude Bernard not only prepared foundations to the theory of discontinuity between the nerve and the neuron, or the nerve and the junction, but also proposed as an option the possibility of a chemical communicant emanating from the nerves.

Actually, during the days of Langley's investigations many findings paved the way – and indeed open the door – to the demonstration of peripheral chemical transmission. First, the findings by Thomas Renton Elliott (1877-1961), Sir Henry Hallet Dale, Walter Bradford Cannon (1871-1945) and others concerning the drug actions and, as proven later, of the sympathetic transmitters at the receptors of the sympathetic autonomic nervous system [13] expanded the concept of receptors from the parasympathetic to the sympathetic system. In fact, Elliott (1904), as stated subsequently by Dale (1934), "advanced… the daring idea that sympathetic nerves liberate adrenaline… to act" on the effectors. Second, there was the work of Richard Harnack (1875), William Gaskell (1886 and 1916; see above), Richard Boehm (1844-1926), Oswald Schmiedeberg (see above, and [12]), Elliott (see above), Reid Hunt (1870-1948), Walter Ernest Dixon (1871 – 1931), L. Hirschmann (1863), A. Biedl (1895), Heinrich Winterberg (1907), Heinrich Fuehner (1917) and others concerning the autonomy, physiology and pharmacology of the autonomic nervous system, including the effects of pilocarpine, atropine and physostigmine (see Holmstedt & Liljenstrand, 1963; Karczmar, 1970, 1986 and 1996). This work constitutes the basis for Dale's and Loewi's epochal demonstrations. Among these investigators Dixon, Gaskell, Elliott, Fuehner and Hunt may be particularly important. Gaskell (vide supra) provided the anatomical understanding of the autonomic nervous system and was an early student of the autonomic actions of muscarine, while Dixon described in detail the inhibitory effect of muscarine on the heart and the antagonism of this effect by atropine. In fact, he went as far as to suggest that muscarine may be the natural transmitter for vagocardiac inhibition (Dixon, 1907; Dixon & Hamill, 1909). Winterberg and Fuhner demonstrated physostigmine's potentiation of the vagal cardiac action; Winterberg showed also its potentiation of the nicotinic action on the heart rate, while Fuhner described physostigmine's and ACh's action on the frog's stomach and leech's dorsal muscle. Finally, Hunt (with Taveau, 1906) described new chemical methods capable of detecting small amounts of choline and demonstrated choline's vasodepressant action; they also showed that after atropinization sometimes choline cause a rise in blood pressure, an action much later explained as the nicotinic ganglionic effect of choline; and they demonstrated that acetylation of choline increase its vasodepressant potency some 100,000 times, a discovery that impressed

[13] Proving the distinction between the sympathetic transmitters, norepinephrine and epinephrine had to wait till the nineteen forties work of Ulf Svante von Euler. The matters of the sympathetic autonomic nervous system are outside the scope of this paper, and only what is directly pertinent to the paper is briefly presented here.

them tremendously and should have launched them upon the discovery of ACh as a transmitter; in fact, according to Dale (1934) Dixon told him that in 1906 he used the extract of the vagally stimulated dog's heart to arrest the beat of an isolated frog's heart; this was an early, modified rendition of Loewi's experiments – unfortunately, Dixon did not publish this experiment! Altogether, "the actors were named, and the parts allotted" as Dale (1938) claimed with respect to Loewi's discovery of the cholinergic, vagal transmission.

Dale could have as readily used this quotation for his own work of the nineteen tens and twenties. Dale was for a year Langley's student at Cambridge. During the most creative part of his life Dale was the head of the Wellcome Research Laboratory in London and then director of the departments of pharmacology and biochemistry of the National Institute for Medical ReseArch He established the presence of ACh in ergot, and this constituted an important basis for the role of ACh as a naturally occurring substance (and a transmitter); he subsequently proved (in 1929) that ACh is present in the animal body. He also demonstrated the very potent bradycardic and vasodilator action of Ach, and he was struck that this potency was associated with the very evanescent character of these actions (Dale, 1914); to explain it, he postulated the presence of an esterase in the blood! He also demonstrated that atropine converts the depressor action of ACh into pressor effect resembling that of nicotine, and he coined the terms "muscarinic" and "nicotinic" to denote these two facets of ACh's action; he also coined the term "cholinergic". His important work with ergotoxine, catecholamines and histamine is outside the scope of this article (see Dale, 1953).

His work with ACh and the catecholamines led the usually cautious Dale to propose not only the presence of sympathetic and cholinergic transmission in the two divisions of the autonomic nervous system, but also to suggest that ACh may be a central transmitter (Dale, 1935); this point will be returned to later, jointly with the discussion of the work of Dale's junior associate and student, William Feldberg. And, later in his life he posited a hypothesis (Dale, 1934) that was later referred to by John Eccles as "Dale's principle" and that stated that the neuron liberates the same transmitter at all of its endings; today, as it was shown that a neuron is capable of liberating more than one transmitter the "principle" should be amended to pronounce that a neuron liberates the same transmitter or the same transmitters at all its endings.

And then came Otto Loewi, a Frankfurter, who performed much of his experimental work at the University of Graz; he became a professor of pharmacology in Graz in 1903 and worked there till the Nazis imprisoned him in 1939 and did not release him till he turned over to them his Nobel prize money (see Karczmar, 1996). During this period Loewi worked on the regulation of the cardiac beat by ions, including NaF [14], metabolism and protein synthesis, nutrition and insulin function. He turned in the 1911-1926 to the autonomic nervous system and the vagal control of cardiac rate. In 1921, he performed his famous double frog heart-double cannula experiment using the technique which he employed previously in another context; according to his memoirs ("nocturnal design"; Loewi, 1960) he dreamt [15] of using this method to solve the problem of the mechanism of vagal action on the heart and he executed his dream the next day! A year later, Loewi (1922) clearly defined his results as proving the existence of a "Vagusstoff". And then, Loewi employed pharmacological and chemical means to identify the "Vagusstoff" with ACh. In his epochal experiment of 1921 Loewi did not use physostigmine, although he employed it before (Loewi & Mansfeld, 1911); he used it later also (Loewi & Navratil, 1926). Actually, the use of physostigmine may have obscured the results and diminish the beautiful simplicity

[14] Loewi's description of the facilitatory action of fluoride on the heart was not among his most important discoveries, but it is close to the heart of this author as he and Kyozo Koketsu studied NaF much later and described its facilitatory (which they defined as "sensitizing") action at the neuromyal junction and at the Renshaw cell (Koketsu & Karczmar, 1966; Koketsu, 1967). We were not aware of and we did not quote, the pertinent Loewi's work and of his use of the term "sensitization". Notabene, Edith Heilbronn, who also studied NaF (Heilbronn, 1965) did not quote Loewi either…

[15] Actually, in his memoirs Loewi states that he dreamt twice on the subject; when he had the dream the first time, he remembered in the morning that he had an important dream, but he forgot its content; he was ready to note down the dream as it occurred again the next night. Sir Henry Dale made an interesting comment on the matter of this dream to Sir John Eccles (John Eccles, personal communication to this author); unfortunately, this author did not check out this matter at the time with either Dale or Eccles, and now it is too late to verify it…

of Loewi's 1921 experiment, but it certainly renders the experiment more easy; indeed, Loewi's public demonstrations of his experiment at numerous congresses were not invariably successful, and the attempts to duplicate it without the use of physostigmine by others (including Theodore Koppanyi and this author) sometimes failed [16].

Loewi noticed the sensitizing effect of physostigmine in his experiments and in those of other investigators; also, he stressed the evanescence of ACh's action when administered to the perfusate or administered i.v., as noticed by Dale and himself, and, with Dale he proposed that ACh is hydrolysed by an enzyme; neither of these scientists use the term "anticholinesterase". Altogether, it was most fitting that, in 1936, Loewi and Dale were jointly awarded the Nobel Prize.

Loewi assumed that ACh driven transmission obtains at other than the vagus parasympathetic sites, as well as non-autonomic sites, and this question was pursued in his laboratory and elsewhere. Thus, William Feldberg, Sir Lindor Brown and others demonstrated that ACh is the transmitter at the myoneural junction, and William Feldberg, A. W. Kibjakov and others established that ACh is released from preganglionic nerves and acts as a transmitter at the sympathetic and parasympathetic ganglia. Also, it was shown that ACh is a transmitter at the parasympathetic nerve endings communicating with salivary gland and the bladder, at the pupillar eye sites (Koppanyi, 1929), at the enteric nervous system (see Gershon, 1981) and, exceptionally, at the sympathetic innervations of the sweat glands (see Sperling & Koppanyi, 1949). It should be noted, that, except for the case of the neuromyal junction, the experiments in question concerned muscarinic sites, whether the investigators in question used or did not use, the term.

4. Eccles's Demonstration of Central Chemical, Cholinergic Transmission and Immediate Post-Ecclessian Studies of Central Nicotinic and Muscarinic Transmission

And then came the expansion of the concept of chemical cholinergic transmission from the periphery to the central nervous system. This expansion was preceded by the studies of actions of the calabar bean and atropine on the CNS; these studies originated with the work of the Edinburgh medical men and toxicologists, described earlier in this chapter, and, of course, antedated by some fifty years the proposals concerning the chemical transmission. So did the work of the followers of the Edinburgh team, such as Heinrich Roeber, Emile Harnack, Charles-Edouard Brown-Sequard (vide supra), William Heubner, Ludwig Kleinwaechter, Albert Bezold with Emile Goetz and others concerning the effects of physostigmine of the bean extract, atropine, curare and jaborandi on the mammalian central nervous system (see Waser, 1953 and Karczmar, 1970 and 2007). For example, Harnack (with Witkowski, 1876), the Edinburghian Fraser and the Americans Roberts Bartholow and John Hudson stressed that the extract (or, physostigmine) caused in animals "central paralysis" and respiratory depression, and that atropine antagonized these effects; these findings constituted the basis for the use of atropine as a treatment for antiChE poisoning; till today, atropine is employed as an antidote of organophosphorus antiChEs. Beginning with the nineteen twenties and thirties, besides measuring such "overt" effects as overt convulsions and respiration, the investigators employed more sophisticated methods such as measurement of reflexes (Schweitzer & Wright, 1937) and first primitive and then advanced forms of the EEG (Makrosian, 1937), and applied drugs in question, including ACh directly to the cortex, intraventricularly (Henderson & Wilson, 1937) and to the spinal cord, particularly after Sjostrand (1937) demonstrated that intravenously applied ACh cannot cross, as a quaternary, the blood-brain barrier (and, which was less readily realized, is hydrolyzed on its way to the CNS, by the ChEs. And, for the first time, Gantt and Freile (1944) and Funderburk and Case (1947) studied the effect of cholinergic drugs (they referred to them as parasympathetic agents) on behavior and conditioned learning. Finally, Joshua Gaddum (Chang & Gaddum, 1933) demonstrated the CNS presence of ACh, while Feldberg showed the spontaneous release of ACh from the animal brain and the facilitation of this release by CNS stimulation; both Gaddum and Feldberg employed a bioassay to show the presence of ACh in the CNS perfusate. Notabene,

[16] Other, interesting circumstances concerning the success of Loewi's experiment are detailed in Karczmar, 1996.

the studies in question were contemporary with the speculations by Dale, Feldberg and Henry McIntosh as to the central transmittive function of ACh, yet, very few of these investigators envisaged the possibility in question or quoted Loewi or the other scientists mentioned (see Karczmar, 2007).

It was already stated that in the nineteen thirties Sir Henry Dale proposed "formally" (see above) that ACh is a central transmitter; Sir William Feldberg, who, after his escape from Nazi Germany was for awhile Dale's student seconded this notion, basing in part his opinion on finding that AChE is distributed unevenly and in an organized fashion in the CNS, which he felt is consistent with the notion of the presence at the sites of AChE localization of chemical, cholinergic transmission (Feldberg, 1945). And then came Sir John Carew Eccles' conversion… Australian born Eccles was in the nineteen twenties a graduate student of Sir Charles Scott Sherrington (1857 - 1952), a neurologist, neuroscientist, philosopher (Sherrington, 1940), a Nobelist and a published poet. Sherrington himself carried out experiments on the spinal cord, defined integrative role of the CNS and stressed the importance of inhibitions for this integration; and he did posit the possibility of chemical inter-neuronal communications. With Sherrington, Eccles worked initially on the neuromyal transmission and its electrophysiology, and the characteristics of this transmission led Eccles to believe, in spite of Sherrington's generalizations, in electric nature of neuromyal as well as central transmissions. In the nineteen forties and fifties, he was not alone in this belief; in fact, during this period most neuroscientists shared Eccles' notion (Eccles, 1964; Karczmar, 2001a and b). Actually, the great Russian neuroscientist, J. S. Beritoff stated clearly at the time that ACh does not play any part in central excitations or inhibitions (see Karczmar, 2007). Eccles' objections to the role of ACh as central transmitter were based, among others, on his belief that ChEs would not be able to terminate the action of ACh at the synaptic sites, ACh would clog the synapses, and fast transmittive potentials were not consistent with chemical transmission. Many believe today that what Dale referred to as Eccles' "conversion" was based on Sir Karl Popper's influence. Popper, an Austrian born philosopher transplanted to UK, visited Eccles during the latter's stay as Professor of Physiology in Dunedin, New Zealand, and stressed to Eccles the necessity for a scientist of flexibility (see Robinson, 2001 and Bacq, 1975). However, there is evidence (see Karczmar, 2001 a and b, and Karczmar 2007; also, Eccles' personal comments to A. G. Karczmar, circa 1990) that, prosaically, Eccles' conversion was due to the demonstration by his friend Steve Kuffler, who was Eccles' associate in Canberra, Australia, of cholinergic transmission at the neuromyal junction; as well as the influence of his friends, including Alexander Forbes, who was, like Eccles, Sherrington's student (see Karczmar, 2001a). Next, following his personal contacts with B. Renshaw (see Karczmar, 2007), Eccles found a favorable, appropriate site where he could prove or disprove the transmittive CNS as he realized that, if the motor terminal at the skeletal muscle releases ACh, then the motor collateral to the Renshaw interneuron must also release ACh, by virtue of Dale's principle (vide supra). And, he became aware of an intra-arterial drug administration method developed by Bo Holmstedt and his associates at the Karolinska Istitutet, and he used it in conjunction with microelectrode technique perfected in Canberra laboratories (see Karczmar, 2007) that could be applied to the Renshaw preparation. Finally, Eccles selected two able investigators and experimentalists, Paul Fatt and Kyozo Koketsu, to attack the problem.

So, the great moment arrived and in a number of studies (the first of which, little known, appeared in 1953 in an Australian journal), Eccles, Fatt and Koketsu showed that the Renshaw cell response evoked by antidromic stimulation of the motor nerve was potentiated by physostigmine and blocked either by the tertiary curarimimetic, beta-erythroidine applied intravenously, or the quaternary d-tubocarine applied directly; they also demonstrated that ACh and nicotine applied directly duplicated the Renshaw cell response elicited via antidromic stimulation (Eccles *et al.*, 1953 and 1954). Short of identifying ACh as being liberated at the collateral [17], this was indeed the first proof of the central presence of a cholinergic synapse! And to add to the beauty of these results, Eccles and his colleague demonstrated also that following the antidromic stimulation the motoneuron shows an inhibitory response

[17] Indeed, the proof of the release of ACh from a single central cholinergic neuron is not available even today, although studies of single neurons were initiated by Ezio Giacobini (Giacobini *et al.*, 1963). Indeed, Giancarlo Pepeu and others (see, for example, Pepeu *et al.*, 1990) showed the release of ACh from activated central cholinergic pathways, but not from single neurons.

that could be blocked by strychnine, a glycine antagonist rather than by nicotinics or curarimimetics: this result established that the Renshaw cell is an interneuron which transforms excitation into inhibition by an inhibitory transmitter (in the CNS, inhibitions can be also achieved directly rather disynaptically).

The presence of cholinergic transmission in the CNS is a tenet *sine qua non* of this article, whence the necessity of describing in some detail the pertinent demonstration, even though this demonstration dealt with the nicotinic, not muscarinic transmisson. But, within years of Eccles' discovery his Canberra colleagues and followers, David Curtis, Rosamond Eccles, John Phillis, John Crawford and others revealed that, while the nicotinic response predominates in the case of the Renshaw cell, the latter exhibits as well a weak, muscarinic response (they developed the electrophoretic technique which they used in their work; Curtis and Eccles, 1958). It was shown later that most central neurons display a double response, generally a primary nicotinic and a secondary, weak muscarinic response.

Actually, initial post-ecclesian work in Canberra, Montreal, Germany, Italy and elsewhere of Chris (Kasimir) Krjevic, John Phillis, David Curtis, John Crawford, Danielle Bovet, Albert Herz, E. Longino, Philip Bradley, Joel Eccles, Nigel Bidsall, Sir Arnold Burgen and others, mostly with electrophoretic techniques seemed to show that a majority of central neurons respond to muscarine and related drugs, and that this response, as wel as that to physostigmine is blocked by atropine (see, for example, Bradley and Elkes, 1953; Krnjevic and Phillis, 1963; see Karczmar, 1976 and 2007); this appeared to be true for many brain parts, including cerebral cortex, the striate, the medulla, and the limbic system, and, in fact, is true also for the neuromyal junction and the autonomic sympathetic ganglia. In fact, the cholinergic correlates of behaviors and function that were studied before Ecclesian era (see above) as well as immediately after Eccles' discovery appeared to be muscarinic in character (see below). The early investigators who worked with the central muscarinic transmission as well as with peripheral muscarinic responses initiated the knowledge of the characteristics of the synaptic muscarinic transmission, particularly with the advent at Canberra of intracellular recording methods. Thus, Syogoro Nishi, Nae Dun, Ben Libet, Kyozo Koketsu Patricia Shinnick-Gallagher, Joel Gallagher, Vladimir Skok, Rosamond Eccles, Nae Dun and this author (see for example, Galagher & Shinnick-Galagher, 1986; Nishi, 1896; see also Karczmar *et al.*, 1986) described the various responses and their physiology of the sympathetic and parasympathetic ganglia; they showed that the responses of the sympathetic ganglia are predominantly fast and nicotinic, and that these ganglia also exhibit slow, muscarinic and extra-slow peptidergic response, as well as inhibitoy catecholaminergic potentials elicited via the chromafin cell. Finally, presynaptic nerve terminal potentials abund as well. This multiplicity of responses and, particularly, the presence of two potent responses, the nicotinic and muscarinic resembles the central response of some central cholinergic cells. On the other hand, parasympathetic ganglia respond mainly nicotinically, and rarely and weakly show a muscarinic potentials, thus resembling some other central cholinergic neurons. Altogether, this early realization of the semblance of the central and autonomic muscarinic responses was well illustrated by a statistical correlation in the mice between the potency of a number of muscarinic cholinolytics (atropine substitutes) as mydriatics (mydriasis being a muscarinic phenomenon) and their potency as protectors against the central lethality induced by the OP antiChE, tetraethyl pyrophosphate (TEPP); this correlation suggested that there is a close resemblance between peripheral and central MRs (Karczmar & Long, 1958).

In parallel with the studies of the synaptic characteristics of muscarinic and nicotinic responses of the ganglia, similar studies were carried out with respect to the CNS, beginning with the work of Eccles (1964) on the ionic transfer that accompanies the muscarinic as well as the nicotinic synaptic transmission. And, studying the CNS – particularly the cortex - Chris Krnjevic (1969) described both the slow muscarinic responses and the fast nicotinic responses as well as their kinetics, and subsequently (see other references to Krnjevic in Karczmar, 2007) their ionic responses; J. S. Coombs, J. E. Desmedt,, R. Lorente de No, John Hubbard, David Curtis and Chris Phillips carried out similar work, as described in John Eccles' fundamental book on central synaptic mechanisms (Eccles, 1964). Altogether, according to this early work, several ions participate in the currents generated by these responses, and the ganglionic muscarinic response is generated by the inhibition of the K^+ current (the M current; Brown & Adams, 1980). Later, David Adams demonstrated the presence of the M current in the central muscarinic responses (see below). The main characteristic of the central muscarinic response, is its metabotropic nature,

contrary to the ion-gated feature of the nicotinic response; this metabotropic nature consists of the coupling of the muscarinic response to guanine nucleotide-binding protein (G protein); while less well documented, it characterizes as well the muscarinic reponse of the sympathetic ganglia (see also below).

5. Muscarinic Receptors and Their Subtypes: from Abstract Notions to the Chemical Isolation, Molecular Biology and Mechanisms of Transmission

It was some 50 years after Langley established the "receptor" concept and some 30 years after Dale referred to the muscarinic autonomic transmission, that for the first time it was shown by Walter Riker and William Wescoe (1951) that an antagonist different structurally from atropine, gallamine, blocks preferentially the vagocardiac transmission, suggesting that the existence of more than one subtype of muscarinic cholinergic receptors (MChRs). And, ten years later, Peter Roszkowski (1961) demonstrated that McN-A- 343, a butynyltrimethyl ammonium derivative had a preferential agonist effect of the sympathetic ganglion muscarinic response, while exhibiting little if any activity with regard to muscarinic smooth muscle and cardiac responses. These finding and the subsequent work of Barlow (Barlow *et al.*, 1976) and Leithold (Leithold *et al.*, 1977), particularly the demonstration by Leithold that pirenzepine discriminates as an antagonist between three receptor subtypes, led to the notion propounded by Sir Arnold Burgen (see Burgen, 1989) and Nigel Birdsall (see Birdsall & Hulme, 1989) that there are multiple subtypes of muscarinic receptors (see also Karczmar, 2007 and Karczmar *et al.*, 2007). It must be noted that the investigations beginning in 1951 of William Paton, Eleanor Zaimis and Agneta Nordberg (see Karczmar *et al.*, 2007) led to a similar conclusion with respect to the nicotinic receptor.

The discoveries concerning the multiple receptor subtypes led to what may be termed the abstract era of the cholinergic receptor lore, that is, the identification of the muscarinic and nicotinic receptors via their structure-activity relationships (SARs) with regard to the actions of their agonists and antagonists, rather than via establishing their chemical structure, the latter task being almost impossible technically prior to nineteen sixties and difficult prior to nineteen seventies or nineteen eighties (see Christopoulos, 2007). The SAR approach became valid after Alfred Clark (1926), long before the establishment of the notion of subtypes of mAChRs, posited the concept of competitive actions at the receptors of agonists and antagonists. And Clark, Joshua Gaddum, the Nobelist Robert Furchgott, Heinrich Schild, William Paton, Sir Arnold Burgen and the Nijmegen investigators Everhardus Ariens and J. M. Van Rossum developed equations reflecting these competitive actions and yielding constants identifying the SARs specific for the various receptor subtypes (see, for example, Furchgott, 1964; see also Karczmar *et al.*, 2007). In fact, at the time Furchgott posited that there will be never a chemical and structural identification of receptors, and of the chemical change that accompanies the receptor-drug interaction [18]! Yet, already in the nineteen fifties, that is, at the apogee of the abstract phase of receptor research, and preceding Robert Furchgott's dictum, indirect evidence emerged concerning the "material" rather than abstract reality of the receptors and their subtypes. Thus, Pater Waser employed radioactive ligands and George Koelle's histochemical stain for AChE to demonstrate the binding of decamethonioum and curarine to the skeletal muscle (intercostals) endplates (Waser, 1953; see also Waser, 1960 and 1983). In this research, Waser was concerned with nicotinic receptors, and his research was followed with that of Lee, Raftery and others (see Lindstrom, 1998) who employed such radioactive toxins as ligands as alpha-bungarotoxin in their work with nicotinic receptors. Soon, however, similar work of Everhardus Ariens, Nigel Birdsall, Edward Hulme and others (see Christopoulos, 2007) with a radiotoxin derived from the snake mamba and radioactive muscarinic agonists and antagonists such as oxotremorine and dexamide to demonstrate the presence and the distribution of MRs.

[18] Robert Furchgott's elegant 1955 review of sympathetic and cholinergic – mostly muscarinic – receptors is more than 80 pages long and contains 404 references; either one of us may hold the record for both categories (see Karczmar, 1967). He earned the Nobel Prize in 1998 for his experimental receptor work and for his theoretical conceptualizations, as well as for his discovery of the nitric oxide as a second messenger. Once, he agreed upon the invitation from the American Society for Pharmacology and Experimental Therapeutics to become the candidate for the ASPET and, inconsistently, told several of us: "Vote for me if you want a bad President"…

As to this author, the real drama occurred at a Rio de Janeiro Symposium that took place in 1959 (the proceedings of this Symposium were published under the editorship of Carlos Chagas and Paes de Carvalho in 1961)[19]: at this Symposium Carlos Chagas with Aida Hasson Voloch and Sy Ehrenpreis employed their own extraction, purification and precipitation methods to obtain two different powders which they presented to the astonished audience as "real" receptors. As pointed out by Arthur Christopoulos (see Karczmar *et al.*, 2007) polysaccharide rather than protein binding constitutes the danger in interpreting whether purification or precipitation results, and the "receptors" of Chagas, Hasson and Ehrenpreis were not the right nicotinic receptors [20]. But, there was a progress with the chase of "real" nicotinic receptors, as Jean-Pierre Changeux employed a more specific purification method to obtain a protein that specifically bound decamethonium (Changeux *et al.*, 1970). And subsequent research of Albert Karlin and Ricardo Miledi (see Karczmar *et al.*, 2007) using still better purification, toxin binding and membrane dissolving methods brought up definitive isolation of the nicotinic receptor. This isolation was facilitated by the availability of several sources very rich in nicotinic receptors, while this is not true with regard to MRs which, furthermore are "unstable in many commonly used… detergents" (Schimerlik, 1989); porcine atria and porcine cerebellum were found to be such a source, and used to isolate the receptors in question successfully, but, as shown by Edward Hulme, Nigel Birdall, Sir Arnold Burgen and others, successful solubilization and isolation of the MRs can be carried out with respect to, for example, rat forebrain (Hulme *et al.*, 1983).

Subsequently, cloning and other molecular biology techniques, NMR methodology used already some thirty years ago by Nigel Birdsall and others, as well as development of additional ligand antagonists and agonists and their radioautography led to further understanding of the spatial structure of the MRs and their active sites, their allostericism and distribution, and of the electrogenesis of the potential change evoked by their stimulaton. These receptors generate, both at the sympathetic ganglia and in the CNS slow excitatory potentials (slow EPSPs, as shown by Syogoro Nishi, Kyozo Koketsu (see Karczmar *et al.*, 1986) and Chris Krnjevic (1993); early, the decrease in membrane conductance that occurs during the generation of the EPSP, was ascribed By David Brown and Paul Adams (1980) to suppression of the so called M-current (see above).

Today, we possess a good picture of the MR and its subtypes; they are glycoproteins and members of the seven transmembrane-spanning, guanine nucleotide binding protein (G protein)-coupled receptor (GPCR) superfamily; their alpha-helical domains are connected by 3 extracellular and three intracellular loops, while their two terminals, amino and carboxyl, are extra- and intra (cytopasmic) cellular, respectively. Major differences between the MR subtypes involve the two terminals and the thirteenth loop. An inner, hydrophilic gorge or pore that exhibits an anionic site, serves as the ligand site, while the thirteenth loop is a major component of the intracellular coupling of the G protein and activation of the MR. Today, 5 MR subtypes (M1 – M5) are generally recognized. While the distribution and contribution to functions and behaviors of the MRs will be discussed subsequently, the detailed discussion of the structural, binding, activation properties of these subtypes and of the electrogenesis of the currents that they generate is outside the scope of this article; at any rate, these subjects will be reviewed in Laerte Oliveira's Chapter of this volume, and they were covered recently in a number of

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[19] Carlos Chagas was the son of Carlos Justiniano Ribeiro Chagas (1879-1934), the discoverer of Chagas disease, and the first investigator to describe in full a new infectious disease as caused by a pathogen, a trypanosome. He became the director of the Rio de Janeiro famous Oswaldo Cruz Insitute. He received numerous prestigious awards, including the Schaudinn Prize, the contenders for the prize including Paul Ehrlich, Ilya Mechnikov and Emile Roux. Twice he was nominated for the Nobel Prize, which however he never received.

[20] Sy Ehrenpreis was, at the time, a fellow in Dave Nachmansohn's laboratory. However, astute Nachmansohn did not accept Ehrenpreis' "receptor", as the "real" nicotinic receptor. Recently, as at the XIIIth International Symposium on Cholinergic Mechanisms, held in Foz do Iguassu, Brazil, this author recounted this story, Changeux, himself Nachmansohn's ex-fellow and his great admirer (Changeux *et al.*, 1985), felt that this account did not sufficiently forcefully represented Nachmansohn's critical view of Ehrenpreis' "receptor", and he publically reprimanded this author (and his very good friend!) quite vigorously.
Well, Sy Ehrenpreis was wrong as to his receptor identification, but he certainly was a brilliant pianist and, at many Meetings or before his own presentations (if a piano was available) he regaled his audience with his playing.

reviews (see, for example, Birdsall & Lazareno, 2005; Christopoulos, 2007; Birdsall, 2009; Brown, 2009).

An interesting feature of the MRs should be emphasized before this specific subject of receptors is left behind. Both in animal development and in ontogenesis, the MRs do not appear simultaneously, in a synchronized matter with other components of the cholinergic system such as ChEs or choline acetyltransferase (CAT); they do not form at a single ontogenetic or phylogenetic moment a mature, functionally ready synapse or junction. Indeed, they appear already in the unfertilized oocytes and in the blastoderm of several species, including fish and mammals (Lammerding-Kopple *et al.*, 1995); in fact, in the case of Xenopus they are present before the appearance of the nicotinic receptors (Kusano *et al.*, 1982). And, they appear, prior to synaptogenesis, in the central nervous system of the chick and of the rat (Enna *et al.*, 1976; Lammerding-Koppel *et al.*, 1995), in the non-innervated early limb buds (Lehmann *et al.*, 1991), developing but not innervated skeletal muscle and muscle cell cultures (Furlan and Godinho, 2005 and Furlan *et al.*, 2009), as well as other developing organs (see Furlan and Godinho, 2005, and Karczmar, 2010). Also, MRs and other cholinergic components are present in non-innervated tissues, such as placenta and glia (see Koelle, 1963). Finally, MRs and other cholinergic components appear throughout phylogenesis, including animal (and plant and bacterial) species devoid of nervous systems and such functions as motility; this is true particularly for cholinesterases (Karczmar, 2007 and 2010). What is the purpose of the presence of the MRs and the other components of the cholinergic system under these circumstances? In some cases, their early preneuregenetic presence is explainable by trophic and metabotropic – facilitating metabolism and ionic movements - capacities (see Karczmar, 2010). In a general sense, the cholinergic components that appear preneuregenetically and throughout phylogenesis must have some *exaptic* role, that is, they must be exploitable and exhibit an evolutionary benefit that is independent and different from that represented by the mature, transmittively functional cholinergic system, the term "exaptation" being coined by the great late evolutionist, Stephen Jay Gould (see Gould, 2002) to explain the phenomenon of "*ex multis unum*", that is, to elucidate the enigma of phylogenetic (and ontogenetic) conservation of traits that only ultimately, by forming a complex system, exhibit the final adaptive capacity (see Karczmar, 2010).

6. Cholinergic Pathways and the Central Distribution of Muscarinic Receptors

It helps in explaining clearly functional and behavioral role of MRs to describe the central cholinergic pathways, as the anatomical sites of these pathways relate directly to these functions and behaviors, as well the central distribution of MRs.

To start with the cholinergic pathways, William Feldberg stated early that central cholinergic sites, as identified by ChE assays are selective, rather than uniformly distributed, and he opined that such a distribution constitutes the evidence for the presence of cholinergic synapses (and pathways) in the CNS. Subsequently, using his (and Jonah Friedenwald's; Koelle & Friedenwald, 1949) histochemical staining method for localizing ChEs (ultimately, Koelle could localize both AChE and BuChE, and adapted his method to its use in electron microscopy) George Koelle described cholinergic localizations in the CNS, stressing their concentrations at such sites as the limbic system, reticular formation and the striatum (see Koelle, 1963). Subsequently, using Koelle's method, Michel Gerebzoff, Peter Lewis and Charles Shute provided a more continuous picture of the cholinergic pathways, particularly with regard to limbic pathways and ascending reticular system.

However, the presence of ChEs, and particularly AChE characterize both presynaptic and postsynaptic sites, as well as non-cholinergic localizations, and cannot be used dependably to identify the cholinergic pathways; but, the McGeers with Henry Kimura developed an immunohistochemical method for detection of choline acetyltransferase (CAT; McGeer *et al.*, 1974), the enzyme that synthesizes ACh; this method is almost as good as would be a method for direct detection of ACh, and much more reliable for establishing the cholinergic pathways

than the technique involving staining of AChE. This method was used very successfully by the McGeers, Marsel Mesulam (Fig. 1), Fibiger, Wenk, Larry Butcher, Nancy Woolf, Bruce Wainer, Paul Kasa and others (see Karczmar, 2007) for delineation of cholinergic pathways (Fig. 1). Their maps were established mainly for rats, cats and other animals, but, according to Marsel Mesulam and others they resemble very much, or are basically identical with, the maps constructed, in fewer cases, for the humans; and, they are quite similar, although the various investigators use different nomenclatures to describe the components of their systems. According to Larry Butcher-Nancy Woolf, the cholinergic pathways radiate from the pontomesencephalon forebrain and its sites such as pedunculopontine nucleus and latero tegmental nucleus to basal forebrain and thalamus, while the forebrain sites such as medial septal nucleus, vertical diagonal band and nucleus basalis of Maynert project to the entire cerebral cortex, hippocampus and amygdala (Woolf, 1997). Marsel Mesulam's reticular formation and its ascending system (which he refers to as Ch5 and 6) are similar to Nancy Woolf's and Larry Butcher's pontomesencephalic system; he defines the nucleus basalis radiation as distributed to the cortex, striatum the limbic system and the neocortex (for the analysis of the differences between the pathways described by various investigators, see Karczmar, 2007).

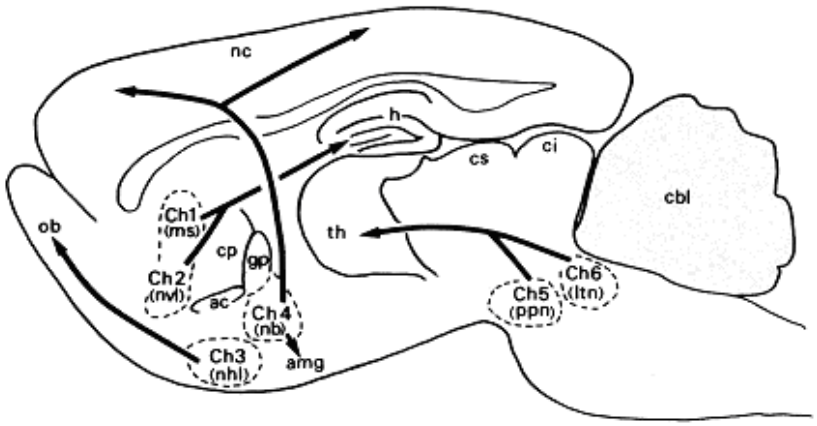

Figure 1: Schematic representation of some ascending cholinergic pathways. The traditional nuclear groups, which most closely correspond to the Ch subdivisions, are indicated in parenthesis. However, the correspondence is not absolute. Abbreviations: ms, medial septum; nhl, horizontal limb nucleus; nvl, vertical limb nucleus; amg, amygdala; cbl, cerebellum; nb, nucleus basalis; th, thalamus; ac, anterior commissure; csB, superior colliculus; ci, inferior colliculus; ppn, pedunculopontine nucleus; gp, globus pallidus; cp, caudate-putamen complex; ob, olfactory bulb; nc, neocortex; h, hippocampus; Itn, lateral dorsal tegmental nucleus. (From Mesulam *et al*., 1983, by permission)

Additional, but still incomplete mapping is based on establishing of ACh-sensitive sites by Chris Krnjevic, Frederic Bremer, Jean Chatonnet, Philip Bradley and others, and, particularly, on the use of the gas chromatography-mass spectrometry method for identifying brain ACh, developed in the nineteen fifities by Don Jenden, Bo Holmstedt and Israel Hanin in Bo Holmstedt's Toxicology Laboratory of the Karolinska Istitutet in Stockholm [21].

As shown above, it is clear that the cholinergic pathways are ubiquitous and that their radiations reach sites important for all functions and behaviors that depend on the brain, and which will be described below. Additional work indicates that there are endless communications between the central cholinergic

pathways and those activated by other transmitters, whether catecholamines, serotonin, histamine or aminergic and peptidergic transmitters (see, for example, McGeer *et al.*, 1987).

To turn now to the central distribution and location of MRs, radioligand binding techniques, quantitative autoradiography, in situ hybridizational histochemistry, selective precipitation by use of antibodies, and position tomography (see, for example, Strijckmans *et al.*, 1997 and Norbury *et al.*, 2004) were used for the identification in question. All 5 MR subtypes seem to be represented in the CNS at the postsynaptic sites, while M1, M2 and M3 receptors are represented presynaptically; for example, the presynaptic locus of M3 receptors was assigned to the striate (Karczmar, 2007; Picciotto *et al.*, 2002); it must be stressed that the activation of the presynaptic MRs results in reduction of ACh release, as well as the release of other transmitters, such as dopamine in the case of the striatal M3 receptors (see Karczmar *et al.*, 1986). While M1 receptors seem to predominate in the cortex of several species, the M1, M2, M3 and M4 receptors may be found in the limbic system, the thalamus, the hypothalamus (particularly in the posterior hypothalamus), the visual areas (cortex) and the cochlea, the forebrain, the locus coeruleus and the pons, and in certain spinal neurons, although the presence of the M3 receptors seem to be low (Christopoulos, 2007).

Altogether, MRs seem to be omnipresent along the cholinergic pathways and as ubiquitous as the latter; it is interesting that the same appears to be true for the nicotinic receptors; in fact, in many cases, both kinds of receptors may be present at the same neurons; their density may be similar, or one or another of the receptors may predominate; for example, in the case of the Renshaw cell, where Eccles' notable research (see above) concerned the nicotinic postsynaptic receptors, MRs are also present, although in small amounts (see above, and Karczmar, 2001a and b, and 2007). This makes the analysis as to the nicotinic or muscarinic nature of functions and behaviors difficult; it is of course even more difficult to assign the receptor basis for a function or behavior with respect to either of the five MR subtypes, or either of the nicotinic receptor subtypes that are more numerous than the MR subtypes.

Another most interesting problem concerns the causality of the emergence of one or another receptor, whether nicotinic or muscarinic, and of their subtypes. As already pointed out, the receptors and their subtypes appear desynchronousky with respect to their innervations and the synaptic differentiation (see also Karczmar, 2010). So, does this specific receptor emergence depend on local conditions that surround the formation of the receptors and their subtypes? Or, is their specificity dictated by the "influences" emanating from the axons or dendrites approaching the postsynaptic sites? At this time, to the knowledge of this author there are very few investigations directed at this issue, and the few studies in question yield ambiguous or, indeed, unexpected results (Karczmar, 2007). And, some receptologists speculate that indeed, the local conditions and their demands regulate this matter (Palmer Taylor, personal communication, 2009).

7. Functions and Behaviors with Cholinergic and Muscarinic Correlates

The cholinergicity of functions and behaviors was established long before the demonstration of the existence of peripheral and central chemical cholinergic transmission; of course, the gap was even bigger with regard to the establishment of the notion of nicotinic and MRs and their subtypes. Thus, as already described, following the introduction of Calabar bean by the Scot missionairies to Edinburgh, Edinburgh medical men, particularly Henry Wadell, Robert Christison and Thomas Fraser demonstrated, in animals and also by self-experimentation, that the bean extracts cause central effects, such as soporific

[21] While presence of ACh in the brain was established in the nineteen thirties and forties by Joshua Gaddum, William Feldberg and others, Jenden, Holmstedt and Hanin were the first to use a chemical method for identification of brain ACh. When they carried out this identification, they sent their results – with a letter, signed by Jenden and Holmstedt only - to Sir Henry Dale, as they assumed that this finding would please Sir Henry. And Dale, with his habitual courtesy, acknowledged, a few months before his death, having received the letter in question.

and hyperthermic effects and respiratory depression and paralysis. In fact, even before their work Hutchinson, the British Consul in the Calabar Province described convulsive action of the bean (see above, and see Holmstedt, 1972 and 2000; Karczmar, 1967 and 2007). And Fraser and other Edinburgh investigators established the antagonism between the bean extract and atropine – all this, without the slightest inkling of transmitter, cholinergic basis of these phenomena! German, British, French and USA investigators continued this work in the first and second half of the nineteenth Century, and Heinrich Roeber provided one of the important findings of this period by demonstrating the analgetic action of the bean's extract when applied to the spinal cord (Roeber, 1868). And the European and American investigators profited from purification of the bean extract and isolation and then synthesis of physostigmine in their further work with eserine or physostigmine and with its antagonist and antidote, atropine (see Karczmar, 2007). Also, they begun to study the effects in animals of muscarine, arecoline and pilocarpine; Oswald Schmiedebrg and Walter Dixon were particularly active, toward the end of the nineteenth century with the effect, central and peripheral, of muscarine (see above; see also Karczmar, 2007; Holmstedt & Liljenstrand, 1963). And, well into the XXth Century an important step was made with the initiation of the studies of ACh. When it became known due to, as already mentioned, to the studies of Sir Henry Dale (1914) and subsequently of T. Sjostrand (1937), that, given intravenously, ACh is readily hydrolyzed and, at any rate, has difficulties crossing the blood-brain barrier to obtain its central effects, ACh was administered into the cortex and intraventricularly (see Koppanyi, 1948 and Karczmar, 2007) and EEG and overt convulsive and twitch-like effects were described; this work was carried even in humans (Henderson & Wilson, 1937).

This work was followed by studies of certain functions and behaviors that are affected by muscarine, atropine and physostigmine; these studies were carried out still prior to the demonstration of the existence of chemical, cholinergic CNS transmission; yet, these investigators referred to the notion of central cholinergic transmission in their interpretation of their results. Thus, Keith and Stavraky (1935) and Gantt and Freile (1944), Moruzzi and Magoun (1949), Bremer and Chatonnet (1949) [22] were concerned with EEG and behavioral seizures and excitations, Mary Pickford (1947) demonstrated cholinergic nature of hypothalamic and endocrine activities, while Funderburk and Case (1947) conducted early animal studies on conditioning and learning. Then, during the early post-Ecclesian era Franco Rinaldi and Harold and Williamina Himwich described arousal and other functions of the ascending reticular system as elicited by cholinergic agents (Rinaldi & Himwich, 1955); Sir William Feldberg with Robert Myers and Steve Sherwood (see Karczmar, 2007) demonstrated via intraventricular injection of muscarinic agonists and antagonists the central cholinergicity of motor activities and temperature regulation; central correlates of aggression were established by L. H. Allikmets and Luigi Valzelli, and Luigi Valzelli stressed the positive effect of muscarinics on aggression, (Valzelli, 1967; see also Karczmar, 1976 and 2007); some ten years later Drachman (Drachman, 1978; Drachman & Leavitt, 1974) stressed, in a series of important publications anteceding the use of cholinergic drugs in senility and Alzheimer's disease the similarity between the action of atropine on the memory of young humans and the characteristics of learning and memory of elderly; and L. H. Allikmets and Carl Pfeiffer (Pfeifer & Jenney, 1957) compared the effect of muscarinics on conditioned response and learning and their positive effect on schizophrenia, an effect expanded much later by this author (Karczmar, 1988; see also Karczmar, 1976, 2007). It should be emphasized that this work and that of others was concerned with muscarinic responses of the CNS and the muscarinic character of several functions and behaviors; In fact, the description of central nicotinic responses of the medial geniculate nucleus by A. K. Tebecis (1970a and b) was quite a rarity at this particular time. The matter changed with the arrival on the scene in the nineteen eighties and nineties of Jean-Pierrre Changeux, Jon Linstrom, Edson Albuquerque, Palmer Taylor and Kenneth Kellar who established the ubiquity of the central nicotinic transmission and their functional and behavioral significance.

Since these investigations, that is since approximately nineteen seventies, incessant investigations concerned the functions and behaviors with cholinergic correlates, and the Tables I and II illustrate the progress made and represent the modern status of our knowledge of this matter; the tables in question

[22] Jean Chatonnet's son Arnaud follows his illustrious father's footsteps as a cholinergiker (see Chatonnet, 2010).

concern these correlates whether they are muscarinic, nicotinic or both in nature. It should be added that it may be doubted whether some of the phenomena referred to as "Functions" in Table 1 warrant the term in question, as in the case of EEG activities and sleep; and, indeed, the classification used is arbitrary.The detailed identification of sources for these tables and the detailed description of the pertinent research is available in Karczmar (2007). Further comments concerning the Tables and their updating, as well as, so far as possible modern identification of behaviors and functions with muscarinic correlates follow.

First, it is obvious from these tables that just about any behavior or function seen in animals exhibits cholinergic, whether nicotinic or muscarinic correlates; this could be readily predicated from the ubiquity of the cholinergic pathways (see Fig. 1). And, the cholinergic functions and behaviors listed here as occurring in animals happen also in humans; of course, animal models of schizoid behavior are just models and do not correspond directly to schizophrenia as human disease; further discuusion of this particular point will appear later. Similarly, while aggression is a human attribute, its classification shown in Table II as predatory may not occur in the modern human. Finally, and importantly, such emotions as happiness and melancholy, and the feeling elicited by experiencing art, including music may exist only and purely in humans; at least, they cannot be measured as yet in animals, and they are not listed in Table II (Sacks, 2009; see however, J. Panksepp's oevre, for example, Kroes *et al.*, 2007).

The functions and behaviors are listed as cholinergic in Tables I and II; their character as such was and is being demonstrated via the use of a number of techniques, and, generally, this listing is not controversial anymore. These techniques include studies of the behavioral and functional consequences of appropriate brain lesions (see, for example, Cuello, 1993; Pepeu & Giovannini, 2004), of the administration of neurotoxins and antibodies, and, more modernly of the knockout, transgenic and cloning procedures (Picciotto *et al.*, 2002; see also Karczmar, 2007); the functional and behavioral responses to localized, microelectrode site-specific injections of whether ACh or muscarinic and nicotinic drugs; pharmacological analysis of functions and behaviors, including the use of cholinergic agonists and antagonists, as well as antiChEs (see Karczmar, 2007 and 2009); and microdialysis techniques, pioneered by Giancarlo Pepeu to establish the release of ACh upon eliciting certain behaviors, such as learning and attention. Furthermore, additional appropriate methodologies were used in evaluation of certain functions and behaviors listed in the Tables.

Similarly, to establish the muscarinic nature of a given behavior or function a number of methodologies may be employed. While the use of atropine and several other non-specific muscarinic antagonists identifies the general muscarinic nature of these phenomena, subtype specific agonists and antagonists may be used to discriminate between subtypes of the M receptors active with respect to a given function or behavior; yet, the presence of all 5 MR subtypes in the CNS and the non-absolute specificity of the effect of selected agonists and antagonists on the five subtypes in question renders this approach difficult. More successful in this respect are the ligand-binding studies, immunoprecipitation techniques with the use of subtype specific antibodies, and in situ hybridization histochemistry (see Christopoulos, 2007; Brown, 2009). As a generalization, all functions and behaviors listed the two Tables exhibit clearly both muscarinic and nicotinic correlates.

7.1. Functions exhibiting cholinergic, particularly muscarinic correlates

To turn now to specific items of Table I: while the reflexes, whether simple monosynaptic reflex, such as the patellar reflex or complex, such as the dorsal root to ventral root (VR-DR) reflex show muscarinic attributes and the M2 presynaptic receptor appears to be involved, the VR-DR reflex responds strongly to nicotinic antagonists (see Karczmar, 2007); the problem here is that the spinal presence and distribution of the M receptor subtypes were little studied. Similarly, anesthesia induced by any anesthetic agent is antagonized by cholinergic agonists including antiChEs such as Edrophonium and facilitated by atropinics, but, it is difficult to assign any particular role in this phenomenon to a specific nicotinic or MR subtype.

Table 1. Functional effects of cholinergic drugs in animals

I. Motor behavior and other related neurological syndromes
- A. Effects on anesthesia and reflexes
- B. Catalepsy
- C. Locomotor and related actions: mobility, gnawing, self-biting, head motion, sniffing; compulsive circling; hypokinesia
- D. Tremor
- E. Convulsions

II. Respiration

III. Emesis

IV. Appetitive (hypothalamic) behaviors
- A. Hunger and feeding: effect dependent on brain site and species
- B. Thirst and drinking; effect dependent on brain site and species

V. Hypothalamic thermocontrol
- A. Heat production
- B. Heat loss

VI. Hypothalamic Endocrine Activities

VII. Cardiovascular Phenomena

VIII. GI Activities

IX. Voiding

X. EEG and brain excitability
- A. EEG arousal and theta rhythms
- B. Synchronization phenomena
- C. Seizures

XI. Sleep
- A. REMS
- B. SWS

XII. Chronobiology
- A. Diurnal rhythms
- B. Hibernation, seasonal changes

C. Aging

XIII. Sensorium
- A. Nociception
- B. Audition
- C. Vision
- D. Olfaction

XIV. Sexual Activity

The motor phenomena that include motility such as kinesia and walking exhibit strong cholinergic correlates, and it is interesting that cholinergic excitation with small doses of nicotinic and muscarinic agonists or with antiChEs induces hypokinesia via their action on reticular formation and elsewhere [hence, the general cholinergic syndrome, the Cholinergic Alert Non-Mobile Bahavior (CANMB) which will be referred to later, includes diminished motility]; nicotinic elements are present in this hypokinesia, M1, M2 (Gomeza *et al.*, 1999) and M4 receptors seem also to be involved, whether in the striate, basal forebrain or reticular formation, while atropine, in small does induces hyperkinesias (see Picciotto *et al.*, 2002; Karczmar, 2007, for references). Larger doses of cholinergic agonists and antiChEs lead to catalepsy, similar to that induced by cannabinoids and morphinoids, or convulsions; indeed, morphinoid and cannabinoid hypokinesias are antagonized by atropine. Finally, tremorigenic actons and parkinsonian-like syndrome, classically induced by oxotremorine (hence the name of this muscarinic agent) in animals appear to involve M2 receptors, also, again, nicotinic effects seem to be present as well; cholinergically induced tremor serves as an animal model of Parkinson's disease. Notabene, sympathetic and GABAergic receptors are involved as well (see Karczmar, 2007).

Control of respiration is a complex matter, involving bulbar respiratory centers, neighboring medullary areas including reticulopontine formation and tegmental areas, baroreceptors of the solitary tract's nucleus, and medullary control of bronchomotor tone, thus causing an interaction between sleep, respiratory activities and vasomotor phenomena (see Thews, 1986; Karczmar, 2007). All these sites, including baroreceptors exhibit cholinoceptive capacities that are mostly muscarinic in character and react to several muscarinic agonists and antiChEs, including insecticides and OP agents; actually, nicotinic agonists and antagonists seem to be less effective with respect to the functions in question. While the identification of the M receptor subtypes was not carried out specifically with respect to respiration, M4 and M2 labelling was obtained all over pontine and medullary areas (see Christopoulos, 2007).

Again, emesis is a complex phenomenon, regulated by the medulary center or chemoreceptor trigger zone, as well as afferents originating in the labyrinthine apparatus (in the case of motion sickness) and in the intestine. The emetic capacity of cholinergic agonists, particularly physostigmine was known since the self-experimentation and other studies of the edinburghian savants in the middle of the nineteenth Century (see above), and studied subsequently by Theodore Koppanyi and Herbert Borison (see Karczmar 2007). The emetic center contains M1 receptors, but it responds also to nicotinic agonists.

Hypothalamus is involved in a number of functions (see items IV, V and VI, Table I). The control of appetitive behavior, that is, activities related to hunger and thirst is located in the lateral, ventromedial and posterior hypothalamus, but it involves also the cortex and limbic sites. The sites in question are mainly muscarinic, and possibly the M3 receptor subtypes are involved in the hypothalamic control of feeding (see Christopoulos, 2007), although other receptor subtypes are present in the pertinent sites of the limbic system. Generally, appropriate muscarinics and atropinics evoke and block thirst and hunger seeking behavior, respectively, but in some animal species the opposite effects may arise, as documented by Robert Myers and Peter Lomax, the pioneers of the studies of the appetitive behavior (see Karczmar, 2007).

Hypothalamus is also the site of thermocontrol, and this control is muscarinically rather than nicotinically mediated. The actual direction of the effect of muscarinic agonists – hyperthermia versus hypothermia - depends on the specific hypothalamic site of the administration of the pertinent agents; while there are only a few studies concerned with the actual M receptor subtype or subtypes involved, the hypothalamic sites involved exhibit M3 receptors. The following comment is pertinent: according to Ernst Gellhorn, Sam Grossman and Robert Myers, the hypothalamus is involved in the Claude Bernard's homeostasis of the "milieu interieur", and this notion suggests that there should be a causative and homeostatic link between energy phenomena and appetitive as well as thermal activities; thus, cholinergically induced heat production or pyrexia should be accompanied by an increase in food and, particularly water intake; this is not always true in all species (see Karczmar, 2007).

Finally, the hypothalamus controls the endocrine activities. Two systems participate in this control. The first system involves supraoptic and paraventricular hypothalamic nuclei that link the hypothalamus with neurohypophysis and control the release of oxytocin and the antidiuretic hormone, the vasopressin. As known since the nineteen forties work of Mary Pickford, cholinergic agonists and antiChEs, applied to the pertinent hypothalamic sites (and, as shown later, into the third ventricle) cause ther release of oxytocin and vasopressin.The second system consists of arcuate and periventricular nuclei which produce release factors that act on the adenohypophysis (anterior pituitary) and liberate pituitary trophic hormones, including ACTH, corticosteroids and weak androgenic hormones. Also parabrachial, reticular, and forebrain systems, as well as magnocellular nuclei, and emotions centered on the cortex and the limbic system are involved. While the endocrine systems seem to be predominantly muscarinic in nature, yet nicotinic responses also can be obtained; thus, vasopressin and oxytocin release may be obtained by nicotinic agonists and blocked by both atropine and hexamethonium (Hillhouse & Milton, 1989). M1 and M2 receptor subtypes are involved in the muscarinic functioning of both systems, and other M receptor subtypes participate in the endocrine effects emanating from the other pertinent sites (see Karczmar, 2007).

Cardiovacular phenomena are a mixed bag as, again hypothalamic as well medullary and limbic sites, nucleus magnocellularis and reticularis dorsalis, substantia innominata and intromediallateral spinal column are involved in these phenomena. And, of course sympathetic and parasympathetic

ganglionic sites regulate heart rate and cardiovascular responses, as do sinocarotid baroreceptors, studied intensely in the nineteen twenties by the Nobelist Corneille Heymans (see Karczmar, 2007). Muscarinic as well nicotinic cardiovascular and cardiac responses may be elicited, and M1 and M2 responses can be evoked; the direction of these effects depends on the site investigated; for example, hypotension and hypertension can be evoked, respectively, by the application of M2 agonists to ventrolateral and posterior hypothalamus (Martin, 1992). And autonomic ganglionic stimulation results in muscarinic vasodilatation of the central arterioles and pial vessels, wheter via smooth muscle or endothelial action; M3 and M5 receptor subtypes seem involved (see Karczmar, 2007).

Gastrointestinal and bladder regulation involves parasympathetic and sympathetic ganglionic control, complex descending and ascending pathways beginning and terminating in the cortex, cranial nerves such as the dorsal vagal complex, additional brainstem sites and pontine and tegmental nuclei (constituting the mictrition center), and spinal pathways studied particularly by William de Groat and his associates (see, for example, de Groat & Yoshimura, 2009). Both nicotinic and muscarinic responses may be obtained, but there is a paucity of data concerning the M subtype receptors that may be involved.

Central EEG and related phenomena are manifold and behaviorally important, particularly as regards EEG and behavioral arousal (or alerting) and theta rhythms, sleep functions, and EEG and overt seizures (or convulsions).

To start with, the forebrain, pontomesencephalic, striatal and retinal neurons exhibit spontaneous rhythmic activities that are obtainable in vitro and that do not seem to depend on sensory input; the pharmacology of these potentials is not clear at this time (see Karczmar, 2007). Then, the EEG patterns include slow rhythms and synchronized pulses, desynchronized, alerting or arousal patterns, and several sleep patterns. The slow patterns include alpha and delta rhythms, the so-called recruitment, theta waves (which accompany the desynchronized or arousal patterns), spindling, augmentation, and post-reinforcement synchronization (PRS). These patterns are evoked by appropriate electric stimulation and perceptions, as well as subconscious events (see below) and are regulated by drugs, including the cholinergic agents; for example, cholinergic muscarinic stimulation blocks spindles and rhythmic firing bursts. They involve reticulo-thalamico-cortico-cerebellar recruitment system, with a contribution from the caudate; P. Andersen and S. A. Andersson were the pioneers in the studies of these phenomena (see their book on alpha rhythms, 1968); the theta waves are discussed below.

Other synchronized, evoked potentials include somatosensory cortical potentials (the P13 potentials of rats and P50 potentials of humans), visual evoked potentials and reversed visual evoked potentials (VEP and PR-VEP potentials), event-related potentials (readiness or 'Bereitschaft" potentials; BP), the P300 and CNV waves, and epicortical potentials or fields such as skilled-performance positivity (SPP; Chiarenza *et al.*, 1990; see Karczmar, 2007). There is a behavioral significance to these patterns; for example, the PRS which can be recorded from the parieto-occipital cortex expresses achieving an expected goal, and it denotes the restoration of human and animal sensitivity to new clues, as established by Tad Marczynski, the pioneer of the PRS studies (Marczynski, 1993). BP potentials underlie certain subconscious and decision-making phenomena, as discussed below, while SPP denote evaluation of results of a goal-oriented activity. These potentials are evoked in various cortical sites and depend on limbic, septohippocampal and nucleus basalis radiations to the cortex. The locations of these circuits within prominent cholinergic pathways and the evidence obtained with lesion experiments and the evaluation of the Alzheimer's disease patients indicate that these potentials are cholinergic in nature. And, the PRS potentials are blocked by atropinics (which convert them into supersynchronous waves; Longo, 1966) and nicotinolytics (which also change them to slow waves of high voltage); it was generally shown that these antagonists block other synchronized and evoked potentials; yet, sometimes cholinergic stimulation obliterated certain slow and evoked potentials, as in the case of thalamic spindles rhythmic bursts; anyway, the pharmacology of the cholinergic involvement in these phenomena is not clear at this time (see Marczynski, 1993) and the subtypes of the M receptors that may be involved were not ascertained.

Desynchronizations, EEG arousal patterns and theta waves are induced by the stimulation of or application of both muscarinic and nicotinic agonists as well as antiChEs to the brain stem including pontine, mesopontine and bulbar reticular formation, gigantocellular tegmental fields, entorhinal cortex,

nucleus basalis magnocellularis, the striate, the limbic system and the hypothalamic formation; the cortical patterns are induced by these various sites via the thalamus, where the stimulation evokes high frequency rhythms. Applied *in vitro* to hippocampal slices and other appropriate preparations, muscarinic agonists and antiChEs produce these EEG and theta phenomena. Whether induced muscarinically (and by antiChEs) or nicotinically, the EEG desynchronization consists of shift of the cortical EEG toward higher frequencies (20 to 30 Hz beta rhythms and 30 to 80 Hz gamma waves) and lower voltages, and it correlates regularly with hippocampal theta waves which exhibit frequencies of 8 to 12 Hz and are higher in voltage. Beginning in nineteen thirties, Giorgio Morruzzi, Frederic Bremer and Horace Magoun studied extensively, as already described, the arousal and desynchronization patterns, and they and Richard Jung, Volia (W.T.) Liberson, F. T. Brucke and Vincenzo Longo, Mircea Steriade (2003) and the Moscow team of Vinogradova and Brzhnik (see Longo and Loizzo, 1973 and Karczmar, 2007) associated desynchronizations and the theta patterns with learning, perception and memory. Evoked by electric stimulation or appropriate drugs EEG desynchronization and its theta waves are similar to behaviorally, sensorily or emotionally evoked arousal, or arousal accompanying attention and learning, although, when the EEG is analyzed by means of long term, mulitchannel analysis pioneered by Don Jenden there are subtle differences between desynchronization evoked by appropriate drugs on one hand and behavioral conditions of the other, (see Fairchild *et al.*, 1975). And, the phenomena in question evoke liberation of ACh from appropriate sites (see, for example, Fisher *et al.*, 1999). It should be emphasized that the anatomic systems involved in the phenomena in question are very complex, as are the behavioral and perceptual consequences of their activation; some investigators, such as A. Wikler referred to the relations between these two sets of events as a "divorce"; see Karczmar, 2007, for further discussion of the"divorce" concept.

It should be emphasized that the slow, synchronized potentials and the slow rhythms, and the desynchronized rhythms are mutually antagonistic; thus recruitment, augmentation, spindling and delta patterns are blocked by stimulations, whether behavioral or cholinergically induced, and converted into desynchronized, low voltage waves; as maintained by Mircea Steriade, Donald McCormick and Alan (J. A.) Hobson (see Karczmar, 2007), the reticular and the dorsal geniculate nuclei – both cholinergic - underlie the antagonism in question. Actually, even earlier, Giorgio Moruzzi, Horace Magoun, Franco Rinaldi and Harold Himwich proposed that there is a dipole between behavioral arousal and sleep, based on the acivities of "ascending reticular system", which corresponds to the systems described by Hobson, Steriade and other investigators discussed above.

Endogenous EEG seizures or convulsions [EEG seizures do not necessarily lead to overt convulsions, that is repetitive muscle contractions and relaxations of either clonic or tetanus (tonic) form] in humans and animals. They may have a febrile or diabetic basis, or be due to drugs, certain types of brain lesions or brain strokes and tumors (see Luria, 1973). Here, the concern is with EEG seizures and overt convulsions induced particularly by cholinergic agonists and antiChEs. In this case, they arise via destabilization of several forebrain loci, such as the limbic system and cortical areas such as the pyriform cortex, possibly via generation of the long-term potentiation (LTP)-like phenomena; the response then spreads to other cortical and forebrain neurons via recruitment and kindling processes (Potier & Psarropoulou, 2004). GABA and excitatory aminoacids are involved in the EEG seizures, but muscarinic, and also nicotinic agents induce these seizures and overt convulsions, and facilitate drug-induced (as, for example, by strychnine) seizures; atropinics and nicotinolytics antagonize seizures induced by cholinergic agonists, as well as electroshock or strychnine; M1 and M2 receptors are apparently involved in the muscarinic induction of seizures, and M1 agonists cannot induce seizures in M1 knockout mice (see Karczmar, 2007); and M2 antagonists prevent induction of seizures in the presence of GABA antagonists. Large doses of carbamate and OP antiChEs act similarly; in their case, one must distinguish convulsions or seizures caused by these agents; cardiovascular and respiratory failure and the resulting anoxia, and convulsions and seizures caused by their direct brain actions; ample evidence indicates that these last parameters are primary reasons for antiChEs-induced convulsions and seizures, including the finding that seizures are induced by these agents in isolated preparations (Lebeda & Rutecki, 1987; see also Karczmar, 2007). Finally, the notion that the waves and the spikes that are characteristic for the EEG seizures may be considered as examples of EEG hypersynchrony is

consistent by the finding that the EEG desynchrony induced by small doses of muscarinic agonists or antiChEs antagonizes seizures evoked by convulsants, including strychnine (Karczmar, 1974).

Now as to sleep (see Table I); the sleep may be divided into two main phases, the slow wave or synchronized sleep (SW) and the rapid eye movement or dream sleep (REM sleep); both phases were recognized – under other names - already by Lucretius and in 1765 Francisco Fontana described the REM sleep as "sonno profundo" (see Karczmar, 2007), but the EEG-based definition of these waves begun with the development by Adolph Beck and Hans Berger toward the end of the nineteenth Century of the EEG techniques (see Brazier, 1959, and Karczmar, 2007; actually, several subphases of sleep are recognized today). Detailed description of the SW and REM sleep were provided as early as in the nineteen thirties by Richard Klaue and some twenty firve years later by Nat Kleitman and his graduate student, Eugene Nasarinsky (Kleitman, 1963; and see Karczmar, 2007).

The SW is characterized by slow delta waves, 1.5 – 2 Hz, 7. 5 microvolt combined with the so-called k complexes and sleep spindles (Rechtschaffen & Kales, 1968). As studied by Hernandez-Peon, Colin Drucker and their assciates (see Kaczmar, 2007 and Warren, 2007) it involves Namba's limbic circuit with the lateral preoptic area and the thalamus, and it is evoked by local application of muscarinic agonists and antagonized by atropinics – there is insufficient knowledge of the M receptor subtypes that control the SW.

Mixed frequency, low voltage waves, as well as pontogeniculate occipital (PGP) spikes, as well as motor atonia characterize the REM sleep; its EEG resemblance to alertness underlies the use of term "paradoxical sleep" with respect to this phase of sleep that occupies some two to three hours of sleep in human adults and more in the infants. Michel Jouvet of Lyons is the pioneer of anatomical, physiological and pharmacological studies of REM sleep, and his work was followed by that of Mircea Steriade, Alan (J. A.) Hobson, Chris (J. C.) Gillin (who studied the various phases of sleep, including REM sleep, in humans) and Rodolfo Llinas (see Karczmar, 2007). The giant cells of reticular formation, lateral dorsal and peribrachial tegmentum, locus coeruleus and additional brain stem neurons, sending their message to oculomotor nuclei participate in the REM sleep desynchronization, while they also activate inhibitory reticular pontine formation and the inhibitory tegmental nuclei, inducing atonia. And, according to Alan Hobson (Hobson *et al.*, 1993) different pontine sites regulate the appearance of immediate but short-lived REM sleep and long-term, delayed REM sleep. Michel Jouvet initially concluded that REM sleep is regulated by "monoamine game", i. e., by catecholamines and serotonin, but later he showed that atropine blocks the REM sleep. Today, it is obvious that the two phases of REM sleep have a cholinergic basis; indeed, REM sleep activities in animals induce cortical and hippocampal release of ACh. And, even when the use of reserpine and other means deprives the animals of catecholamines and serotonin, antiChEs and cholinergic muscarinic agonists still induce the EEG image of REM sleep (Karczmar *et al.*, 1970). This cholinergicity of the REM sleep is muscarinic in nature and REM sleep deprivation upregulates the M receptors; the MI, M2 and M4 receptors are involved (see Karczmar, 2007). The REM sleep is associated with creative processes and building of new associations, as indicated by the consequences of its deprivation; for this, and additional reasons Karczmar (1995; see also below) speculated that an alert syndrome of the awaken animal and human which he referred to as cholinergic alert non-mobile behavior (CANMB) represents an "awaken phase of REM sleep".

Chronobiology (see Table I), a science pioneered by Franz includes diurnal, seasonal and oestral phenomena. The retinal-hypothalamic pathway terminating at hypothalamic suprachiasmatic and paraventricular nuclei is in light-sensitive diurnal patterns, as established by the Nobelist Julius Axelrod (see Karczmar, 2007), and hypothalamus and limbic system regulate other forms of time cycles. And, parasympathetic sensitivity may cycle seasonally (Friedman, 1971). There are many data concerning cycles in ACh, AChE and CAT levels and ACh release that may correspond to these rhythms; unfortunately these data are inconsistent (see Karczmar, 2007). Also, circadian rhythms are lost in Alzheimer's disease, perhaps concomitantly with the loss of cholinergic neurons in this disease. The functions of suprachiasmatic nucleus are interrupted by nicotinic blockers, while the data concerning muscarinic contributions to this activity and to the light-induced rhythms are inconsistent (Dunlap *et al.*, 2004; Karczmar, 2007).

The great Russian cholinergiker, Mikhail Michelson (1974) opined that there are no cholinergic correlates of sensorium. This view was refuted, as sensory circuits contain important cholinergic sites

and pathways; these cholinergic pathways originate in the forebrain and abut upon retina, olivocochlear bundle, medial vestibular nucleus, olfactory bundle, pedunculopontine projections and somatosensory including auditory and visual cortex, as well as associative and integrative brain areas including geniculorecipient and superior colliculus areas. Also, projections from the nucleus isthmi to retinotectal areas and the retinotectal and reticulogeniculate afferents are involved in visual perception. Some of the sites listed release ACh, most exhibit M and N receptors (as, for example, the retinal amacrine and ganglion cells; Keyser *et al.*, 2000). And, both muscarinic and nicotinic agonists and antagonists exert effects upon these structures; for example, they facilitate sensory evoked potentials in the sensory cortex and antiChEs, and ACh and nicotinic and muscarinic agonists mimic the inhibition by the olivocochlear bundle of the responses of the auditory nerve terminal, although the effects of nicotinolytics and atropinics were inconsistent; also, cholinergic regulation of the ganglion cells is involved in the discrimination between light and light-off signals, (see, for example, Katz *et al.*, 2004; for further details see Karczmar, 2007 and 2009). Finally, M1, M2, M3 and M4 receptor subtypes seem responsible for some of the sensory activities, and M1 receptor subtype of the cortex seem to be needed for the sensory cortical responses (Zhang *et al.*, 2005). It is also noteworthy that there exist light-generated, endogenous photochromic ChE inhibitors which participate in visual activities (Straschill & Perwein, 1975).

Finally, analgetic phenomena, although sensory in nature, are also part of the behavioral animal and human armamentarium and will be discussed below.

The last item of Table I concerns the sexual activity. Ultimately, this activity (such as erection of both the clitoris and the penis) depends upon lumbar parasympathetic outflow, and is thus muscarinic. Of course, this activity is linked with higher central loci that include hypothalamus, limbic system and the cortex. These sites and their electrophysiological including EEG expression and evoked potentials are facilitated by muscarinics and antiChEs; thus, early Mary Pickford and her assscociates evoked overt sexual behavior by administrating DFP and physostigmine to hypothalamic sites (see Karczmar, 2007) which are muscarinic in nature, and their activity correlates with the animal courting behavior (lordism, solicitation, "attractive" overt behavior, etc.); M1, M2 and M3 receptor subsites are involved in these phenomena (see, for example, Kow *et al.*, 2004, and Karczmar, 2007). And, R. G. Heath injected ACh intraseptally in humans (such procedures were allowed to be carried out in humans in the nineteen seventies) and obtained EEG "orgastic'" pattern undistinguishable from that observed during authentic orgasm (see Figures 9-24 and 9-25 in Karczmar, 2007); unfortunately for the biased cholinergikers, norepinephrine induced similar EEG…The nicotinic involvement in sexual activities is uncertain and controversial. It should be emphasized that cholinergic activities, including the courting behavior are also under central regulation at the cortical, limbic and hypothalamic steroid receptor sites by progesterone, estrogens and androgens (Karczmar, 2007).

7.2. Behaviors and mental activities with cholinergic, particularly muscarinic correlates

As in the case of functions, most behaviors or mental activities that could be observed and/or measured in animals or humans exhibit strong, sometimes preponderant cholinergic, and indeed, muscarinic correlates (see Table II for essentially animal behaviors). Yet, some mentalizations were measured or discussed in human, but their existence or measurability in animals is unsure. Thus, as already indicated, musicophelia that may be considered as an emotion in the case of humans (and emotions are listed in Table II) was presumably not studied in animals, and its cholinergic correlates, if any are not known; and Jack Panksepp may have initiated measurements of happiness in animals (see above), but these studies are at their dawn, and certainly nothing is known about happiness' cholinergic correlates. And what about feelings or emotions of selfishness and altruism, and other moral attributes of the humans? Again, under another name they may be studied in animals (see below), but it is still too early to be definite about this problem, and certainly the involvement of muscarinic receptors in the moral phenomena is unknown…

Table 2. Behavioral psychological and "mental" functions with cholinergic correlates

I. Aggression
A. Emotional (affective)
B. Predatory
C. Irritable
D. Territorial defense
E. Frustration, etc.
II. Learning and Related Phenomena
A. Conditioning
B. Memory (short and long term)
C. Habituation
D. Retrieval
E. Attention and exploration
III. Emotional Behavior and Fear
IV. Addiction, dependence, withdrawal syndrome
A. Opiate addiction
B. Alcoholism
C. Cocaine addiction
D. Nicotine addiction
E. Cannabinoid addiction
V. Analgesia
VI. Social Behavior and Imprinting
VII. "Schizoid" behavior
VIII. Organism-environment interaction (OEI)
IX. Cholinergic Alert Non-Mobile Behavior (CANMB)
X. Subconscious behavior
XI. Cognition versus the "I"
A. Self-awareness

Animal and human aggression present many endogenous forms, such predatory aggression or defense of the pups in animals, and emotional aggression evoked in crowds by inflammatory oratory in humans. And, in animals, there are many models of aggression used to study its physiology and pharmacology, such as emotional aggressions induced by isolation (a model preferred by Luigi Valzelli, a pioneer of aggression studies) or by foot-shock, and territorial or pup-defense aggressions observed in pseudo-natural, ethological habitat, the "Mouse City", a model developed by Charles Scudder (see Karczmar *et al.*, 1973), and aggression induced by drugs, including cholinergic agonists. There are strong cholinergic correlates of all these models, and it must be emphasized that, while catecholamines, paticularly dopamine given i.v. or applied to the limbic system may induce aggression, yet they or their antagonists do not affect some or many of the aggressions expressed in the models in question. And, there is much additional evidence as to the cholinergicity of aggression. For example, the sites involved in aggression as indicated by consequences of their pharmacologic or electric stimulation – such as amygdala and the limbic system, the hypothalamus, thalamic nuclei, nucleus basalis, tegmental nuclei, insular and cingulated cortex, dorsal periventricul-periaqueducal region, and ventral striatum - exhibit cholinergic pathways; then, these sites release ACh upon induction of aggression; "killer" rats exhibit higher levels of CAT and ACh in amygdale and other pertinent sites, as compared to non-aggressive rats; rats bred for high cholinergic supersensitivity or for high levels of cholinergic components exhibit markedly elevated aggression; and cholinergic, particularly muscarinics, applied systemically or to the pertinent sites induce or facilitate aggression, while atropinics block it (see Karczmar, 2007). More difficult to interpret is the "rage" and aggression (Kluver-Bucy syndrome) elicited in humans by damage (accidental) to the septum and in animals following septal lesions; however, aggression is regulated via

the balance between inhibitory (GABAergic) and excitatory circuitry and between various limbic and hypothalamic sites, and the lesions in question may be a consequence in shifting this balance toward aggression. Finally, while effectiveness of nicotinic agonists and antagonists is controversial (see Karczmar, 2007), the mucarinic basis of the various forms of aggression is clearly documented; judging by the cholinergic pathways involved and the known distribution of the M receptor subtypes several M receptor subtypes may be expected to be involved in aggression.

The cholinergic contribution to cognition became of great general interest since the early findings by David Drachman that, as evidenced by specialized quantitative tests the deficit of memory and learning in elderly is identical with the deficit evoked by atropine in young population; his preliminary data suggested also that the two deficits, atropine-induced in youth and endogenous deficit exhibit by elderly may be antagonized by physostigmine (see above). And further interest in cholinergicity of cognition was raised by the evidence that cholinergic neurons are lost in the course of Alzheimer's disease, this loss contributing to Alzheimer's cognitional impairment, and by the indication that cholinergic agonists may ameliorate this condition. Altogether, an immense investigational effort was directed at processes of memory, learning and related states, and, by this author's estimation, some 20,000 papers are published yearly in this area!

Of course, the cognitive research in humans is of great significance, but animal research and animal models for the study of cognition are very important; these models are manifold. They include simple conditioning studies of acquisition, extinction and habituation components of cognition, in which auditory, visual, olfactory, taste and other reward clues are used and simple blink or motor response constitutes the unconditioned response, operant behavior evocation being necessary for learning. Then, there are more complicated models that include continuous trials involving escape, reward and several operant behaviors, as in the case of Sidman continuous trial paradigm. Non-operant tests include the use of mazes and water mazes, alteration tests as in the Y-test, radial and multiple arms mazes, "place learning" and spatial orientation tasks, and testing of the response to a change in reward or punishment, or to a novel paradigm. It is easy to increase the complexity of the tests and the length of the recall period. Finally, ethological and field research are used with respect to several animal species including apes (see Picciotto *et al.*, 2002 and Karczmar, 2007 and 2009). Interestingly, some of these tests may be employed, including operant models, in a modified form in humans, and more complex cognitive tasks and evaluations that involve questionnaires, optical models concerning spatial and composition evaluation, and EEG and evoked responses are used in humans.

These tests, whether in humans or animals, uncovered a number of types of memory and learning, including early learning versus memory consolidation, short term and long term memory, storage, recall or retrieval, and "shallow output memory", that is "the memory of an event … that is not placed in its proper spatiotemporal context" (Cipolotti & Moscovittch, 2005). Furthermore, a number of cognitive states are involved or needed, or antagonistic with regard to exertion of memory and learning; these states include attention, vigilance, arousal or alerting; exploration; vigilance; neophilia or novelty seeking; extinction; and habituation and dishabituation.

The distinct cholinergic, mostly muscarinic nature of these cognitive processes relates first of all to their dependence on known cholinergic pathways and sites. These include radiations from the nucleus basalis and reticular formation to the cingulated and other neocortical areas, the tegmental nuclei, the limbic system and its hippocampus, and dorsomedial striate. Their correlation with cognition was confirmed by the deficits in cognition that is caused by the use of neurotoxins such as kainate, immunotoxins and particularly the cholinotoxin AF64A that exerts more specific actions on the cholinergic synapses than other neurotoxins (see Hanin, 1990), and by pertinent lesions; in fact, accidental injury or pathologic damage of these brain areas in humans cause cognitive deficits. Then, learning and memory processes are correlated with increase of brain and blood levels of ACh and its release from pertinent brain sites; the latter was shown via microdialysis techniques by a prominent pioneer of cholinergic studies, the Florentine Giancarlo Pepeu (see for example Pepeu & Giovannini, 2004). Next, pertinent lesions and the learning and memory deficits that they cause may be remedied by using the growth factors, as shown by another important cholinergiker, the Montrealese Claudio (A. C.) Cuello (1993). Furthermore, nootropics, the drugs that facilitate cognitive processes without being muscarinics,

nicotinics or antiChEs aument ACh turnover and release and increase CAT levels in pertinent brain parts (see Picciotto *et al.*, 2002 and Karczmar, 2007, 2009 for additional evidence as to the cholinergicity of cognition). Significantly, in animals nicotinic and muscarinic agonists and antiChEs facilitate, nicotinolytics and, particularly, atropinics antagonize learning and memory and block nootropical facilitation of cognition; actually, these agonists improve memory in aged adult humans and, to an extent, in AD patients. It must be added that states that accompany or facilitate cognition, such as attention and novelty seeking may be specifically facilitated by muscarinics and antiChEs (Pepeu, 2010). Considerable evidence, including the use of knockout mice, suggests that M1 receptor subtypes are involved in cognition; they also may affect specific states needed for cognition, such as attention (Giovannini *et al.*, 2003; see also Karczmar, 2007), although there is some controversy in this respect (see Picciotto *et al.*, 2002); M3 and M5 receptor subtypes may be involved as well (Picciotto *et al.*, 2002).

Several general theories on the mechanisms of memory and learning, and generally of cognition were advanced. Sir John Eccles suggested in the nineteen eighties that dendritic sprouting and neuritic growth underliescognition, as they are facilitated by learning processes (see Karczmar, 2007). More recently, evidence was obtained by Paul Layer and Willbold (1995), Mona Soreq and others that neuritic and axonal growth is induced by AChE or certain AChE variants (see Karczmar, 2010). Another hypothesis refers to long-term synaptic potentiation (LTSP) that occurs during activation of cortical, hippocampal sites and results in increased release of ACh; this LTSP is enhanced by muscarinic and nicotinic agonists (see Karczmar, 2007).

Altogether, today the predominantly cholinergic nature, perhaps especially muscarinic, of memory and learning, and, indeed of cognition is accepted universally [as proposed in the nineteen sixties by Karczmar (1967), David Drachman and others; see also Bartus *et al.* (1982)], and Mesulam (2000) stressed the importance of cholinergic cognition in evolution. This cognitive significance of the cholinergic system is a component of Karczmar's hypothesis of the CANMB behavioral and cognitive syndrome, already mentioned; this matter will be again discussed later.

Human schizophrenia and schizoid behavior of animals may be considered, in a simplification, as aberration of cognition and of the conception of reality. Several animal models of schizophrenia are available, including amphetamine and atropine-induced animal "psychosis", emotional consequences of inescapable shock and related CER models, and results of the loss of aggressive encounter (Karczmar, 2007). An interesting model is the "no-goal" behavior, developed by N. R. Maier and studied extensively by Volia (W.T.) Liberson (see Liberson & Karczmar, 1969); in this case the rats are caused, via a certain paradigm, to develop, when confronted by a problem which is punishable if not solved, a "fixated", yoked behavior pattern even though they "know" that there is an answer for the problem. Cholinergic, particularly muscarinic agonists antagonize and atropinics aggravate the animal schizoid behaviors, as, sometimes do tranquilizers and anxiolytics; it is of interest that the "no-goal" behavior is not affected by tranquilizers but is attenuated by the muscarinics (W. T. Liberson and A. G. Karczmar, unpublished data; there is another significant component of the test in question, the "inspiration" moment, that will be considered later). Interestingly, in 1957 Carl Pfeiffer evoked a moment of lucidity giving a muscarinic agonist to advanced hebephrenic schizophrenics (Pfeiffer & Jenney, 1957); twenty years later it was shown that when schizophrenics treated with certain neuroleptics were given atropinics to combat the dyskinetic side actions of their treatment, the effectiveness of the treatment was reduced (Singh & Kay, 1979; see also Karczmar, 1988). There is, however, contrary evidence (see, for example, Sarter *et al.*, 2005), and some data implicate other than cholinergic transmitters in the etiology of schizophrenia (see, for example, Guidotti *et al.*, 2005).

The interesting studies of animal social and related behaviors are just in their infancy; available data suggest that some of them may be muscarinically evoked or facilitated and antagonized by atropinics. Thus, imprinting in birds is reduced by atropinics, as are several social activities of mice, scuh as mutual grooming, contact and maternal care, as studied in the model ethological environment, the "Mouse City" (see above). These activities were also facilitated by physostigmine, and when they were compared between mice strains, the strains with higher brain ACh levels exhibited higher incidence of

the activities in question (C. L. Scuder, A. G. Karczmar and G. Kindel, unpublished data; see also Karczmar, 2007).

Addictive behavior concerns opioids, alcohol, cocaine, nicotine and, perhaps, cannbinoids (marijuana). Operant and conditioning behaviors may be used in animals in the pertinent studies. For example, in the case of the paradigm developed by Alfred Kahn (Karczmar *et al.*, 1978) certain strains of mice that show alcohol preference will seek alcohol even if its delivery is available at the mice's non-preferred or normally avoided site, or when this delivery is linked with overcoming of the footshock. Meso-limbic reward-punishment dipole, nucleus accumbens, hypothalamus and tegmental pathways are involved, and these sites and pathways are essentially cholinergic. Furthermore, in the case of all these addictions, cholinergic parameters are affected in the course of their development; thus, there may be a release of ACh from pertinent structures, and increase in ACh or CAT levels, although these levels may return to normal once addiction is formed, as in the case of opiate addiction. And lesions to the punishment-reward axis affect or prevent the formation of certain addictions (Picciotto *et al.*, 2002). These parameters generally change during withdrawal, but, in this case, the anxiety or stress may be the cause (Karczmar, 2007). Furthermore, depending on the addiction, there may be sensitization or upgrading of either muscarinic or nicotinic receptors. Finally, particularly in the case of nicotinic addiction dopaminergic mesolimbic system is strongly involved. While much of this evidence suggest that increased cholinergic tonality may increase in addiction, a rather unique, contrary opinion was delivered by Janowsky (Janowsky *et al.*, 1989).

Cholinergic analgesia was described long before the demonstration of cholinergic transmission, whether at the periphery or in the CNS: it was mentioned by the Edinburgh investigators (vide supra), and, when purified bean extract, or physostigmine became available, many investigators of the nineteen thirties showed its occurrence in humans and animals (see Karczmar, 2007). In fact, the French medical men, Jacques Tinel and his associates (1933), described that intraventricularly administered ACh relieved the causalgia of the hand! Today, the nociceptive studies are carried out by means of several methods usable in animals, and some techniques that can be employed in the humans; hot plate test and tests depending on evocation of various responses such as tail flick, flexing, rearing and vocalization responses, and response to certain stimulations of forebrain structures are commonly used (see Karczmar, 2007). As indicated by lesion experiments, electric stimulation, local application of cholinergic agonists, etc. (see Karczmar, 2007) pain signals are mediated by spinal (dorsal horns and spino-thalamic tracts), as well as brain sites that include periaqueductal gray, reticular areas, hypothalamus, basal ganglia, rostral ventro-lateral medulla, several thalamic and hippocampal nuclei, nucleus Raphe magnus, nucleus cuneiformis and thalamico-cortical pathways; the pathways and sites in question are cholinergic. This suggests that analgesia is cholinergically induced; in adddition, the cholinergicity of nociception in human and several – but not all, see below - animal species is indicated by evidence that includes the apropriate use of neurotoxins, including AF64A, cholinergic agonists, antagonists and hemicholinium, the antagonist of choline uptake, and antiChEs (the cholinergic drugs are analgetic in these species whether given i.v., intraventricularly or applied to pertinent central and spinal sites); and increase of ACh levels, its release and turnover follows analgetic stimulation (see Karczmar, 2007).

An interesting finding is that of analgetic synergism between the use of cholinergic agonists and opioids; this effect was obtained by many investigators (see, for example, Beilin *et al.*, 2005; there are some conflicting data with regard to this synergism). This finding may suggest that cholinergic agonists induce analgesia via inducing the release of endogenous opioids or increasing their penetration into the spinal cord and/or brain, and it was also suggested that cholinergic analgesia depends on the cholinergic interaction with noradrenergic, GABAergic and/or peptidergic systems (see Karczmar, 2007). There is evidence that contradicts this notion in the case of opioid-cholinergic interaction; for example, atropine antagonized cholinergically but not opioid-induced analgesia (see, for example, Koehn & Karczmar, 1978 and Koehn *et al.*, 1980). Still another interesting finding is that while cholinergic analgesia can be readily demonstrated in humans, rodents, amphibia and several other species (in fact, in amphibia cholinergics are potent analgetics, while morphinoids are only marginally effective), the cholinergics do not seem to evoke analgesia in primates; paradoxically, atropine seems to be antinociceptive in primates (Karczmar, 2007).

While both nicotinic and muscarinic receptors participate in cholinergic analgesia, muscarinic responses predominate; M2 receptors seem to be primarily involved, as antinociception is impaired in M2 knockout mice (see Picciotto *et al.*, 2002), but M1 receptors participate in cholinergic antinociception as well (Dussor *et al.*, 2004).

Emotional behaviors that can be readily measured in animals include fear, fear-related tonic immobility and stress, anxiety and aversive response. They generally are accompanied by salivation, lachrymation, urination and defacation (SLUD syndrome), as well as changes in cardiac rate and blood pressure; to an extent, these effects are similar to fear-related signs in humans, and whether in humans or animals they depend on cholinergic phenomena due to activation of the sympathetic and parasympathetic ganglia. This activation is controlled by the higher centers that include limbic system, the hypothalamus, several medullary sites, including the nucleus of solitary tract, and perceptive and associative cortex; again, these centers are rich in cholinergic synapses. Accordingly, fear-like and related behaviors or emotions release ACh (see for example Mizoguchi *et al.*, 2001). And cholinergic agonists, mainly muscarinics, effects of nicotinics being less consistent, facilitated these effects and atropinics blocked it.

Vocalizations are related to emotional behaviors, such as courtship and mating in birds, rodents and amphibia, although they serve also as components of learning and cognition. The pathways involved, as established by appropriate recordings from brain sites, lesion consequences and anatomical studies of afferents to central vocal control nuclei, include several forebrain areas and magnocellular nuclei. There is a paucity of cholinergic studies of vocalizations, but these sites are cholinergic and, indeed, cholinoceptive stimuli, particularly of muscarinic nature, evoke vocalizations (see Monka Sadananda's, 2004 bird studies, and Robert Schmidt's unpublished investigations of cholinergic effects on the various types of amphibian calls; see also Schmidt, 1984).

8. Organism-Evironment Interaction (OEI) and Cholinergic Alert Nonmobile Behavior (CANMB)

The foregoing stresses the cholinergic, indeed the muscarinic nature of cognition, including attention, vigilance and novelty seeking behaviors, as well as social, perceptual (sensory) and emotional activities. Indeed, in many respects, the totality of these phenomena, including the related EEG and evoked potentials resemble, metaphorically, an awaken form of REM sleep, REM sleep being still another cholinergic event. The syndrome in question is referred to by this author as Cholinergic Alert Nonmoble Behavior (CANMB; Karczmar, 1979, 1995, 2007 and 2009). And, it appears from the foregoing that the neurotransmitter nature of the CANMB involves several subtypes of the M receptors.

As the CANMB includes also the cholinergic antagonism of animal schizoid behavior, it underlies the cholinergic significance for reality-seeking capacity. This capacity, cholinergic contribution to sensorium and its facilitation of attention and novelty-seeking acivity suggests that CANMB is of utmost importance for organism-environment interaction (OEI). Altogether, the CANMB with its cognitive component is vital for goal-directed, cognitionally positive, rational human and animal existence!

It must be added that, the evidence reviewed by several modern investigators, including Jean-Pierre Changeux, Paul Layer and this author (Karczmar, 2007, 2009, and 2010) suggest that components of the cholinergic system, including the cholinergic receptors is an ancient system that already co-existed with the appearance of the first animal forms. During its long evolution the system developed many evolutionarily adaptable features ("exaptations", according to Stephen Jay Gould; see also Karczmar, 2010) which, at the evolutionary and ontogenetic maturity of the system combined to perform in the CANMB manner.

9. Subconscious Phenomena

This exciting and puzzling field was analyzed and studied in depth by Sigmond Freud, Carl-Gustav Jung and their followers in a way widely different from the methods used in studying and, particularly, quantifying cognition, aggression, etc., in humans and animals and, indeed, the subject of

Jungian and Freudian investigations differs from those of studies described here. Today, however, subconscious activities are studied in humans and animals, including subconscious, sudden initiation of certain activities and subconscious spatial direction of motor activities, subconscious semantic associative processes, spontaneous alteration of behavior patterns, subconscious perception and learning, "inspiration" moment in the course of the fixated behavior, and subconscious activities which preced the free will (see Karczmar, 2007 and 2009). Thus, H. Shevrin and his associates (Shevrin *et al.*, 2002, Bazan *et al.*, 2007) developed a number of evoked potential techniques to follow in humans the dynamics of the subconscious clarification of concepts and perception ability which appear to be amenable to appropriate pharmacological studies (but still constitute pharmacologically a virgin territory). In the case of Maier's and Liberson's "no-goal" behavior, described above, most of the yoked or "fixated" rats continue in their "no-goal" behavior, punished indefinitely; however, some very unique individuals change their "fixated" behavior via inspiration as it were, and embrace the winning, rewarded behavior pattern. It may be speculated that this "inspiration" relates to subconscious correct learning of appropriate clues; the frequency of the occurrence of the "inspiration" episodes increase with the use of antiChEs and a muscarinic agonist, and disappears with the use of small doses of atropine (W. T. Liberson and A. G. Karczmar, unpublished data).

Then, there is the free will paradigm. In this case, Ben Libet (1993) [23] employed the readiness ("Bereitschaft") cortical potential (vide supra) to demonstrate in humans the awareness and free will phenomena; the readiness potential when evoked either by peripheral or thalamic stimulation appears some hundreds of a millisecond before conscious experience of the stimulus and of the awarenes of the volitional (free will) act, while awareness and volition (or free will) in turn occur prior to motor act; this and additional evidence led Libet to opine that the two delays represent neuronal activities needed for awareness and the volitional, free will act (there is some controversy with regard to Libet's findings; see Karczmar 2007 and 2009).

Altogether, inspite of H. Shevrin's, Roger Nitsch's (see Karczmar, 2007), W. T. Liberson's and Ben Libet's pioneering work, the cholinergic nature and, all the more, the identity of M receptor subtypes involved in the subconscious phenomena is still largely unknown…

10. The Self-Awareness or the "I"

Self-awareness, the self or the "I" - or the "soul", to use the preCartesian and Cartesian nomenclature (see Snell, 1933) - is possibly the most important state for a human, as it is synonymous with his/her sense of existence; indeed, the "self" or the soul were a matter of analyses and propositions since neolithic times (see Karczmar, 2009; unpublished data). Yet, the concept of the "self" is difficult to define. In making an attempt at defining this state, this author follows closely the definition posited in the Encyclopedia Britannica and in several pertinent recent articles in the Wikipedia. This author's definition posits that the "I" is constituted by the sense and the subjective experience of one's identity that is present at every moment of one's existence, without reference to the past or to the future; it constitutes a conversion of thoughts (or cognition, or feelings), memories and ideas into the subjectivity of the moment and of the "self". And, It is important to recognize that the "self" is very different from cognition: the latter is a processs of conscious thoughts and includes the processing of information, learning, attempts at knowledge and conceptualizations, and quantitative, objective analyzing of the cognitive elements.

A perennial argument concerns Cartesian dualism, although it antedates Decartes by millennia (A. G. Karczmar, in preparation). The dualists ascribe a different nature to the soul or the "self" and the body or the brain, while the reductionists propose the notion that the "self" or the soul is a brain-dependent phenomenon. Today, the reductionists seem to be in ascendancy. Yet, it should be remembered that no less a thinker than William James opined that "consciousness" is the name of nonentity" (James, 1950). And, there were important dualists in the recent past, as for example, Sir Karl Popper and Sir

[23] With Rosamund Eccles, Sir John Eccles' daughter, Benjamin Libet was a pioneer, in the nineteen fifites,, of the studies of ganglionic potentials and their transmitters. His important contribution to central phenomena and "free will" are a novel excursion for Libet.

John Eccles (see Popper & Eccles, 1977), as well as more recently, in the case of Freeman Dyson and De Grandpre (1999). This author would like to add that many modern reductionists concerned with mentalizations and the "self" do not recognize the difference between the "self" and cognition, and their argument for the single nature of brain and the "self" is weakened by their use of illustrations concerning neurophysiological basis of cognition as evidence for as similar basis of the "self" (Karczmar, 2009) [24].

An extreme reductionist view is that of John Searle who opined that "consciousness is a system level biological feature just as digestion, or growth, or secretion of bile are system-level, biological features… consciousness is a feature of brain" (Searle, 2006) [25]! More neuroscientific is the view of Jean-Pierre Changeux, Francis Crick and many others (see Karczmar, 2009 and in preparation) who argue vigorously that complex circuits that include those concerned in cognition and memory, in perceptions and emotions provide, via "reverberating" processes, a "non-linear transition … from unconscious to conscious and then to "subjective" and "self"- like processes, yet they do not offer, as it appears to this author, an explanation for this transition. Indeed, this transition appears to him to constitute a real gap that requires a conceptual "jump" to be bridged.

Such a jump may have been provided by Roger Penrose, famous British cosmologist and explainer, with Stephen Hawking, of the "black holes" phenomenon. In several books (see, for example, Penrose, 1994) he speculates that in the course of certain quantal phenomena, the collapse of quantal coherence leads to the moment of "objective reduction" (OR); at this moment there is the conversion of quantal phenomena or quantal world into the macrocosm of our reality and of our world. Furthermore, Penrose, with Stuart Hameroff, Jack Tuszynski, Nancy Woolf and their associates (see Tuszynski, 2006) assign as the site of this process the complex interactions that may represent biological activity which is quantal in nature. These interactions occur at the membranes of neurons that are present in the cortex and elsewhere (in the retina, for example); these interactions occur between the membranal microtubules and several microtubule-asociated proteins (such as microtubule-associated protein 2, MAP-2) and their activation by kinase-related phosphorylations.

Nancy Woolf explored in several investigations (see, for example, Woolf, 1997 and 2006) this notion with respect to neocortical neurons where cholinergic transmission is partcularly strong. These neurons, when muscarinically (not nicotinically!) activated exhibit a messenger cascade involving MAP-2 and dephosphorylations, these phenomena resulting in Penrose's quantal OR. Indeed, it was posited by several authors that the sites in question are involved in regulation of what they referred to as "consciousness" phenomena; however, the consciousness referred to appears to be cognition rather than the "I".

Indeed, Jack Tuszynski's and Nancy Woolf's approach may be a jump from the brain platform to the status of the "self", yet, the argument provided does not appear to this author commonsensical in its translation of the quantal phenomena into the "self" events, or for that matter, the MAP-2 phenomena into the "I". Part of the problem is, of course, that at this time the measurement of the "self" is in its infancy; indeed, available tests – whether valid or not (see Karczmar, 2009) - were generally not employed by reductionists, including Nancy Woolf, Jack Tuszynski and their associates. Furthermore, this author argues that the attempts of the reductionists of relating the brain to the "self" lack logical strength. Indeed, if the concepts of many modern epistemologists and logicians such as Kurt Goedel, Rudolph Carnap, Gottlobe Frege and others were applied to the matter at hand, it could be argued that a system as complex as the brain may not be totally complete in a sense of being able to explain or define every single phenomenon that it exhibits (for details of this argument, see Karczmar, 2007 and 2009, and

[24] The Eastern, particularly the Buddhist philosophy offers a valid insight into the matter of the "self" and the inner being. The Buddhist investgators approach this matter and the study of the "self" via a self-study and other techniques that are different from those employed by modern neuroscientists and philosophers. Although many attempts were made to bridge the Eastern and Western approaches, this matter is beyond the scope of this article.

[25] Searle seems to follow faithfully (without being presumably aware of his source) the German zoologist Karl Christoph Vogt (1817-1895) who claimed that "the brain secretes thoughts, as the stomach secretes gastric juice, the liver bile, and the kidneys urine".

Karczmar, in preparation). In a somewhat similar vein Jeremy Hayward claims that, within a complex sytem such as the brain, there may exist distinct "domains" which differ in their methodologies, and the postulates posited within the "domain" of cognition or consciousness are irrelevant within the "domain" of the "self (see Karczmar, o. c.).

We may lack today tools, whether methodological or epistemological to handle the dilemma in question; indeed, at this time we cannot present as a unified picture of electromagnetic and gravity forces and quantal mechanics (Albert Einsten's dilemma!). So, since we do not understand as yet the physical world, reducing the "self" to the brain and to the physical world and specifying the mechanics of the "self" today is perhaps premature (see Karczmar, 1972).

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