Thomas A. Ban: Neuropsychopharmacology in Historical Perspective

Collated 25

Joseph Knoll: The Discovery of the Enhancer Regulation in the Mammalian Brain and the Development of the Synthetic Enhancer Substances

Knoll	February 4, 2016	Review
Warnes	May 19, 2016	Comment
Knoll	February 16, 2017	Reply 1 to Warnes comment
Knoll	July 14, 2016	Reply 2 to Warnes´ comment
Warnes	August 24, 2017	Response 1 to Knoll's replies (1 & 2)
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Warnes	April 26, 2018	Response 3 to Knoll's response 2
Knoll	May 3, 2018	Additional response 2 to Warnes' response 2
Warnes	August 30, 2018	Final response 4 to Knoll's additional response 2

Joseph Knoll: The Discovery of the Enhancer Regulation in the Mammalian Brain and the Development of the Synthetic Enhancer Substances

A chance to significantly improve the quality and prolong the duration of human life

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Abbreviations

AD	Alzheimer's disease
AMPH	Amphetamine
BPAP	R-(-)-1-(benzofurane-2yl)-2-propylaminopentane
CAE	Catecholaminergic activity enhancer
CNS	Central nervous system
DA	Dopamine
DAE	Dopaminergic activity enhancer
DATATOP	Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism
DEP	(-)-Deprenyl
DMI	Desmethyl-imipramine
EEG	Electroencephalogram
INHN	International Network for the History of Neuropsychopharmacology
IUPAC	International Union of Pure and Applied Chemistry
IUPHAR	International Union of Basic and Clinical Pharmacology
MAO	Monoamine oxidase
MAO-A	A-type monoamine oxidase
MAO-B	B-type monoamine oxidase
MDD	Major depressive disorder
MET	(-)-Methamphetamine
NAE	Noradrenergic activity enhancer
NE	Norepinephrine
PEA	β-Phenylethylamine
PD	Parkinson's disease
PPAP	(-)-1-Phenyl-2-propylaminopentane
SAE	Serotonergic activity enhancer
SAR-study	Structure-activity-relationship study
SER	Serotonin

Introduction

I started my behavioral studies in the beginning of the 1950s to find an explanation why only a minority of the mammalian species is easily trainable and to answer the question why human beings are the most manipulable living organisms on earth.

The ability to acquire an irrepressible urge (acquired drive, active reflex) for a goal not necessary for survival of the individual or species represents the most sophisticated function of the telencephalon. Though the development of an acquired drive always originates in one way or another from an innate drive, which relation later becomes unrecognizable, ***nothing exists in the brain without a rational origin*** (Knoll 2005)

With the discovery of the "glass cylinder seeking drive" (Knoll et al. 1955; see inhn, org, Definitions), we hit upon the right method to study in rats the process of fixing an acquired drive (an *"active reflex"* see inhn, org, Definitions) (Knoll 1956).*The finding that mice are unable to fix an acquired drive* (Berta Knoll 1961) advanced the working hypothesis that the last step in the evolution of the mammalian brain was the development of species with the ability to fix acquired drives (Knoll 2005, 1.3 and 1.4).

Obviously, once upon millions of years ago, mammalian species with ***limited*** capacity to fix acquired drives, the tamable, domesticable species came into being. It remains for the future to learn which mammal was born first (and when?) with this capacity. *Homo sapiens sapiens*, the only surviving human species and the single one with ***unlimited*** capacity to fix acquired drives, thus the most manipulable and creative creature, was born not more than about 143-144- thousand years ago.

I summarized in 1969 the results of my first *16-year research period* in a monograph. Larry Stein, one of the reviewers, keenly recognized the problematic circumstances in communist Hungary in the 1960s (see inhn.org. Books, Joseph Knoll How selegiline slows brain aging, Comment 4, on all three books by Joseph Knoll, September 18. 2014). During this period of my research, I was profoundly compelled to devise a nomenclature with regard to the rubric of Pavlovian reflexology (Knoll J. *The Theory of Active Reflexes. An analysis of Some Fundamental Mechanisms of Higher Nervous Activity.* Budapest/New York: Publishing House of the Hungarian Academy of Sciences/Hafner Publishing Company; 1969; Review of the book – inhn.org; Books, February 27, 2014).

A *36-year research period* clarified, thereafter, those key important brain mechanisms which determine the life of mammalian species and whose members, being capable to fix acquired drives, possess a brain with a higher level of manipulability than mammalian species

whose cortex is missing this potential (Knoll J. *The Brain and Its Self*. *A Neurochemical Concept of the Innate and Acquired Drives*. Berlin/Heidelberg/New York: Springer; 2005. Review of the book: inhn.org; Books, January 23, 2014). This early monograph summarized the quintessence of the enhancer regulation in the mammalian brain.

The main message of this monograph is that the appearance of the mammalian brain with the ability to acquire drives ensured the development of social life and ultimately led to the evolution of human society. This most sophisticated form of organized life on earth is still in the trial-and-error phase of its development. It seeks to outgrow the myths-directed era of its history and arrive at its final state, the rationally directed human society.

To study the formation of an acquired drive the "glass-cylinder-seeking drive" was fixed into the brain of rats. Based on an unconditioned avoidance reflex (escape from a hot plate) and using the sound of a shrill bell, playing the role of a high-priority conditioned stimulus, rats were trained to search for and jump to the rim of a 30-cm-high glass cylinder open at the bottom and top with diameters of 16 cm and 12 cm, respectively, and with a side opening through which the rats (up to 350–400 g body weight) could go inside. The best-performing rats acquired the glass-cylinder-seeking drive in a stable manner, possessing thereafter this acquired drive for a lifetime. These rats showed the same high-grade adaptability and readiness in overcoming different obstacles while reaching the goal as the ones influenced by innate drives, such as hunger or sexual desire.

These experiments confirmed that the faculty for acquiring a drive is uncommon in the animal kingdom. Vertebrates can be divided into three groups according to the mode of operation of their brain: (a) those which operate with innate drives only (the majority), (b) those with an ability to acquire drives (a minority), and (c) the only one living today which operates almost exclusively on the basis of acquired drives (*Homo sapiens sapiens*).

With the evolution of brains capable of acquiring drives, species appeared whose members could manipulate each other's behavior and act together. This was the condition *sine qua non* for the evolution of social living, a form of life that enabled the social group to qualitatively surpass the performance of the individual. It goes without saying that the training of skills to act in concert radically improved the quality of life.

With the development of the human brain, a functional network with *over 100 billion interrelated nerve cells and 10^{11} bit capacity* arose. With this network, and with the inexhaustible capacity of the human brain to acquire drives, from the operation of which conscious perception is inseparable, life on earth reached the most sophisticated form of appearance. Furthermore, human beings, primarily social creatures, are building blocks in the creation of a gigantic product: ***human society****, which represents the highest form of life on earth*.

Decades of studies into the nature of the development of an acquired drive revealed the following:

In the mammalian brain capable of acquiring drives, untrained cortical neurons (Group 1) possess the potential to change their functional state in response to practice, training, or experience in three consecutive stages. Namely by getting involved in (a) an extinguishable conditioned reflex (ECR) (Group 2), (b) an inextinguishable conditioned reflex (ICR) (Group 3), or (c) an acquired drive (Group 4). The activity of the cortical neurons belonging to Groups 3 and 4 is inseparable from conscious perception. In any moment of life the self is the sum of those cortical neurons that have already changed their functional significance and belong to Group 3 or 4.

Metaphorically, every human being is born *tabula rasa* with a telencephalon that resembles a book with over 100 billion empty pages (untrained, naive cortical neurons, Group 1), and with the capacity to inscribe as much as possible in this book throughout life. In reality, cortical enhancer regulation – the modification of the presently still unknown chemistry of the cortical neurons through learning, aiming to establish cooperation between cortical neurons previously unacquainted with one another – is the essence of human life.

Whenever a drive is acquired, chains of ICRs are fixed, and neurons responsible for emotions are also coupled to the integral whole, thus cognitive/volitional consciousness is necessarily inseparable from an affective state of consciousness. The mechanism that binds emotions appurtenances to any chain of ICRs is of crucial importance to interpersonal communication. Cortical neurons belonging to Group 3 or 4 continuously synthesize their specific enhancer substance within their capacity. This means that even in the vigilant resting state (leisure), in the absence of a dominant drive, as well as in the non-vigilant resting state (sleeping), the cortical neurons representing the totality of the already fixed ICRs and acquired drives are permanently under the influence of their specific enhancer substance.

Although the level of this *permanent, undulating activation* remains low, it is unpredictable as to when any group of cortical neurons will be influenced by enhancer substances on the level already inseparable from conscious perception. Thus, ***it is always unforeseeable what suddenly comes to our minds.*** As the totality of the cortical neurons belonging to Group 3 or 4 works continuously on an unconscious level, there is a steadily operating, chaotic background noise in the human telencephalon which *can never cease to exist, but it never endangers the function of the dominant innate or acquired drive.* From this situation it follows that ***rational brain activity is necessarily amalgamated with irrational brain activity and we are in every moment of our life in readiness for experiencing the coexistence of order and chaos in our brain.***

Thus, (a) whenever a chain of ICRs is fixed in the human brain, the proper cortical neurons remain, on an unconscious level, constantly active for life, and (b) if the proper method is used, even a chain of ICRs that had never been recalled after fixation can be activated to the level needed

for conscious perception at any later date. The recalling of any chain of ICRs is necessarily inseparable from an affective state of consciousness, due to the emotions coupled as appurtenances to the cortical neurons when they learned to cooperate with each other. Freud developed empirically sound methods for recalling forgotten chains of ICRs in humans, decades after their fixation.

All in all, (a) past experiences are irreversibly fixed in neurons belonging to Groups 3 and 4 that learned to cooperate with each other and constitute an integral whole and (b) proper stimulation of the cooperating neurons as an integral whole allows the fixed information to be recalled any time later. This is inseparable from conscious perception, and thus the past experience is vividly re-lived in a cognitive and affective manner. (c) Even though it is true that during the operation of a dominant drive the activity of the individual is primarily focused on reaching the goal represented by this drive (rational activity), the ability to simultaneously consciously revive past experiences that are outside the limits of the dominant drive actually operating (irrational activity) is a natural endowment of our brain.

Because of the theoretically immense variability of cortical enhancer regulation, any trial to develop a compound that will reasonably stimulate learning in general seems to be, from physiological point of view, a hopeless undertaking. The natural method of behavioral modification – by means of experience, training, or practice – will likely remain not only the most effective, but the only viable way to change the performance of the cortical neurons in species capable of acquiring drives, and this presumably forever. Therefore everything depends and will in all probability always depend, on teaching, learning and education.

As the background noise in the brain is never interrupted and can even become more accentuated during sleep than in the vigilant resting state, the dream world, the classic example of a man-created universe, has always given inspiration to art. Its ultimate explanation awaits the resolution of scientific problems: the chemistry of cortical enhancer regulation and the natural law that determines the operation of "**The Brain and Its Self**" and is responsible for the immense variability of human activities.

Human society – the maintenance of which has always required the proper manipulation of the brain of its members – still finds itself in a state of development. It seeks its final equilibrium: namely, that state in which behavioral modification induced by the family/school/society triad will be based, from birth until death, on the ***exact knowledge of the neurobiological mechanisms that determine human behavior****.*

In this way, members of the community will understand that the simultaneity of order and chaos in their brain is the physiological reality that determines human activity, and will consciously try to find the acquired drives that optimally fit their natural endowments.

For the time being, those lucky enough to acquire the best fitting drives in due time, in the early uphill period of life, have had fair chances for success and happiness. In contrast, those who for any reason have missed this opportunity will remain frustrated and look for "ersatz". It seems reasonable to conclude that order and chaos are of equal importance in our brain.

Without the ability to adapt ourselves to the concrete (science), we would not be able to survive, without the ability which allows detachment from the concrete and explorations in the infinite (art), life would not be worth living.

The existence of a variety of animal species with extremely restricted abilities to fix ICRs and acquire drives marks Nature's long road of experimentation with the brain. The end result of this process, the human brain, has been the most perfect variety.

The *limitless capacity of the human cortex to fix ICRs and acquire drives* allowed, in conjunction with the development of language, an unmatched interpersonal communication. This unique facility made the cognitive/volitional and affective states of consciousness of the human brain, and as a consequence of it, human social life, unparalleled. Because animals lack similar developments, there is no way to obtain direct evidence regarding the nature of their psychic experiences. Nevertheless, the observation of the goal-seeking behavior of trained monkeys, dogs, horses, dolphins and so on furnishes convincing indirect evidence that the operation of ICRs and acquired drives is inseparable, even in animals, from an archetype of consciousness.

The billions who remained during history untouched by wartime mass killings of their innocent peers and were ready to die in the name of "God", "Fatherland", "King" and so on, illustrates the consequences of the practically unlimited capacity of the human brain to fix acquired drives. Even in the dark history of mankind, the Holocaust – the extermination of millions within a few years with unprecedented success, due to a systematically planned and executed evil mass manipulation of a whole nation (in the 20th century!)– was a unique event.

For example, the Jewish population in Europe amounted before the Holocaust to 9,797.000 of which the killing machinery annihilated 5,860.000. Just to illustrate what this means, let us take Warsaw as an example. In 1939, the population of the Polish capital amounted to 1.3 million of which 375.000 was, in the majority indigenous, Jewish population. Today, among the 1.7 million inhabitants of Warsaw the estimated Jewish population amounts to only a few thousand. It is difficult to confront the issue that even today leaders of important countries openly declare their firm resolution to "Annihilate Israel". ***These horrifying examples further demonstrate the unlimited potential to misuse the physiological endowments of the human cortex on the highest level.***

It is worth to consider that after the extinction of Pithecanthropus erectus, Sinanthropus

pekinensis, Roanthropus dawsoni, Homo heidelbergiensis, Homo sapiens fossilis, etc., Homo sapiens sapiens is the unique, surviving member of the human family. ***Humanity is born with a brain without any knowledge as to how the real world and the human body and mind are functioning. In contrast, we all are born with a brain capable to create a "non-existing world".***

Thus, Homo sapiens sapiens created the still operating myths-directed society. However, in order to survive, it was compelled to discover how the real world is functioning. To the end of the 18th century, general knowledge progressed to a critical level, and since the age of enlightenment, science and technology developed from strength to strength. ***Mankind approaches rapidly the final state: the rationally organized human society, grounded fully on scientific knowledge****.* Only a future global change in education, one based on the exact knowledge of the brain mechanisms responsible for the manipulation of human behavior can finally lead to rationally directed society and terminate the present day era where one hand destroys what the other hand has created.

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This essay elaborates upon the recently discovered enhancer regulation in the mammalian brain, primarily the enhancer regulation of the catecholaminergic brain engine. We already succeeded to transform, via the lifelong maintenance on DEP, a catecholaminergic activity enhancer (CAE) substance, innate low performing, shorter living rats into high performing and longer living ones. *The main aim of this essay is to attest by documentary evidence upon the possibility of an improved quality and longer duration of human life with preventive medication with CAE substances and the opportunity to prevent or delay the onset of age-related neurodegenerative diseases, such as PD or AD.*

PART 1

Indications of the key role of (-) deprenyl (DEP)) in the discovery of the enhancer regulation in the mammalian brain

1.1. The origin of the "enhancer-regulation" concept. We may remember that an eagle pounces upon the chosen victim with lightning speed. Reacting accordingly is a life-and-death matter. Both the attacker and the victim have a split second to respond. This promptness of activation in assault/escape behavior inspired my working hypothesis in the mid-1980s that an unknown, life important enhancer regulation capable to momentarily increase neuronal-excitability might operate in the mammalian brain. Since the cerebral catecholaminergic machinery is responsible for the general activation of the cortex, it was reasonable to expect that the catecholaminergic brain engine must be endowed with this capacity. (-)-Deprenyl (DEP), developed in the early 1960 and became famous in the early 1970s as the first selective inhibitor of B-type monoamine oxidase (MAO), was the key experimental tool which enabled the revelation of the enhancer regulation in the catecholaminergic machinery in the mammalian brain.

1.2. Development of (-)-deprenyl (DEP) the first selective inhibitor of B-type monoamine oxidase (MAO-B) and the first catecholaminergic activity enhancer (CAE) substance. The careful analysis of the nature and physiological significance of the acquired drives directed my attention to the catecholaminergic brain engine which plays the key role in the activation of the cortex. In case we needed to stimulate the catecholaminergic neurons, we used the best disposable experimental tools at the time, the long-acting synthetic PEA-derivatives, amphetamine (AMPH) and methamphetamine (MET). Amphetamines were problematic because as soon as the dose surpassed the 1-2 mg/kg level, the drug-induced continuous, irresistible release of catecholamines from their intraneuronal stores in the brain-stem neurons resulting in aimless hypermotility, thus blocking purposeful behavior. In 1959, I decided to perform a structure-activity-relationship (SAR)-study trying to get rid of this undesirable effect.

In the early 1960s, MAO inhibitors represented a new type of central stimulation, so I began the SAR-study with MET containing a propargyl-group attached to the nitrogen. This group was known to form a covalent binding with the flavin in MAO and irreversibly block the

enzyme. Out of a series of newly synthesized, propargyl-group containing patentable MET derivatives, E-250 (later named deprenyl) was selected as the most suitable for further development (Knoll et al. 1964, 1965).

Selegiline/(-)-Deprenyl (DEP), registered in more than 60 countries and marketed world-wide under more than 100 trade names, is used to treat Parkinson's disease (PD), Alzheimer's disease (AD), major depressive disorder (MDD), and is also a popular anti-aging drug.

Unfortunately, DEP is widely known and famed for being the first selective inhibitor of MAO-B, whereas it is neglected as the first and still the only catecholaminergic activity enhancer (CAE) substance in clinical use. With my third monograph (Knoll J. *How Selegiline/(-)Deprenyl Slows Brain Aging.* Bentham e-Books; 2012; inhn.org; Books, September 5, 2013) I aimed to motivate clinicians to consider that PEA is a native CAE substance and explain how selegiline, a PEA-derived synthetic CAE substance **and** selective inhibitor of B-type MAO, slows brain aging.

The age-related decay of cerebral PEA, due to the progressive increase of MAO-B activity in the aging brain, and dopamine, due to the better than average decline in the dopaminergic neuronal activity during the post-developmental phase of life, are biochemical lesions of aging and the speed of deterioration in behavioral performances depends on the pace of these lesions. DEP, as a synthetic, long-acting PEA-derived CAE substance, maintains the catecholaminergic neurons on a higher activity level, and as an inhibitor of MAO-B, increases the supply of PEA and dopamine in the brain, thereby counteracting the aging process.

In our first longevity study my colleagues and I treated 2-year-old male rats, three times weekly, with 0.25 mg/kg DEP until death. This study has proven that DEP-treated rats preserved their learning ability longer, lost their ability to ejaculate later, and lived longer than their placebo-treated peers (Knoll 1988; Knoll et al. 1989).

When I decided to perform this study, I did not know yet that the dose of DEP which I selected in the early 1960s to completely block MAO-B activity in the brain and is still used world-wide in animal experiments (0.25 mg/kg) and in human therapy (10 mg/day), ***also exerts in this dose its non-specific CAE effect. The specific enhancer effect of DEP is elicited in a much lower, 0.001 mg/kg dose (see Part 1.9).***

Due to its CAE effect, daily maintenance on a low dose of DEP keeps the activity of the catecholaminergic neuronal system on a higher activity level. None of the drugs in use today to increase catecholaminergic neuronal activity exert an enhancer effect. The enhancer

substances, PEA-derived DEP and tryptamine-derived BPAP, are synthetic analogues of native enhancer substances and act accordingly. They are simply transforming the inherently lower performing catecholaminergic neuron to a better performing one, and like their native parent compound, do not change the environmental milieu of the enhancer-sensitive neurons when administered in the specific enhancer dose-range (Knoll 2012, Chapter 9).

In our second longevity study we selected out of a population of 1,600 male rats the 94 sexually lowest performing (LP) males and the 99 highest performing (HP) rats. We treated 44 LP rats with saline and 50 HP rats with DEP. The saline-treated LP rats lived 134.58 ±2.2 weeks, their DEP-treated peers lived 152.54 ±1.36 weeks, as long as the selected saline-treated HP rats (151.24 ±1.36 weeks) (Knoll 1988; Knoll et al. 1994).

Thus, maintenance on DEP transformed the lower performing rats into higher performing ones. Experimental and clinical studies with DEP strongly support the proposal that preventive administration of a synthetic enhancer substance during post-developmental life could significantly slow the natural decay of behavioral performances over time, prolong life, and prevent or delay the onset of aging-related neurodegenerative diseases, such as PD and AD. Since DEP is presently the only world-wide registered CAE substance, a properly designed clinical trial with sexually mature healthy volunteers to measure its anti-aging effect is highly warranted.

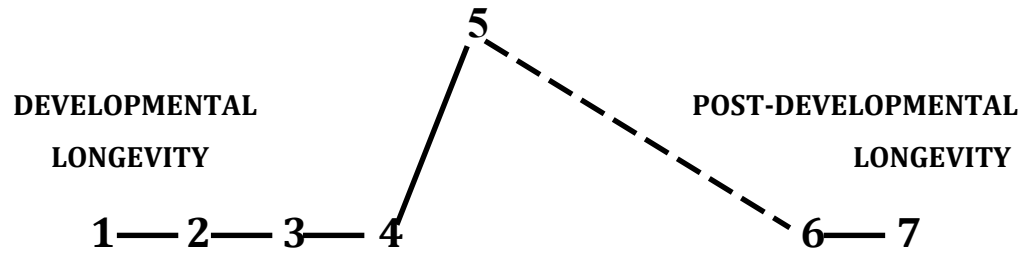
I fully agree with Hayflick who mentioned in his "Theories of biological aging" that "It is possible that only by increasing lifespan, or maximum age of death, of members of a species, will important insight be made into the aging process" (Hayflick 1985). The average human life span surpassed 80 years at the end of the 21st century, but the human technical life span (around 120 years) did not change. In our second longevity study the innate high performing (HP) rats treated with DEP lived 185.30±1.96 weeks, significantly longer than their salt-treated peers, and out of the 50 rats 17 lived longer than the maximum lifespan ever observed during a long observation period on hundreds of untreated or saline treated rats in the strain of Wistar Logan males used in our studies.

The 20th century clarified the physiological significance and exact mechanism of neurotransmission. Today we possess thorough knowledge regarding the nature of the main transmitters and modulatory mechanisms. Their impact on the quality and duration of human life is well known. The history of the enhancer regulation is in its beginning. Up to the present, we published, in detail, only our results with the enhancer-sensitive catecholaminergic and serotonergic neurons. The widely distributed enhancer regulations in the mammalian brain continue to be focus of our studies.

1.3. Congruous data conforming to the concept that quality and duration of mammalian life primarily rests upon the inborn efficiency of the catecholaminergic neurons, "the engine of the brain." There are good reasons to assume that it is the physiological role of the catecholaminergic neurons to keep the higher brain centers in a continuously active state, the intensity of which is dynamically changed according to need. Such regulation is the condition *sine qua non* for the integrative work of the CNS. The catecholaminergic system's operation is comparable to an engine which is ignited once for an entire lifetime, as signaled by the appearance of the EEG, in an early phase of development.

Due to aging, the maximum level of activation of the CNS, via the catecholaminergic system, progressively decreases over time. The blackout ("natural death") of CNS's integrative work, signaled by the disappearance of EEG, occurs when the catecholaminergic brain engine's ability to activate the higher brain centers sinks below a critical threshold, and an emergency incident transpires. This happens when a high level of activation is needed to survive, and the CNS can no longer be activated to the required extent. This would explain why a common infection, a broken leg, or any other normally surmountable challenge given catecholaminergic machinery at full capacity may cause death in old age (Knoll 1994).

The essence of this hypothesis is depicted in Fig.1. According to this scheme, the mammalian organism's life can be divided into seven stages of which six is beginning with a qualitative change of crucial importance.



1) FUSION OF THE SPERMATOZOON WITH THE OVUM
2) THE INTEGRATIVE WORK OF THE CNS SETS IN. APPEARENCE OF EEG
3) BIRTH OF THE FETUS
4) WEANING
5) SEXUAL MATURITY IS REACHED
6) THE INTEGRATIVE WORK OF THE CNS BLACKS OUT. DISAPPEARANCE OF EEG. 'NATURAL DEATH'
7) DEATH OF THE LAST CELL

Fig.1. Conception about essential changes during the lifetime of mammals

The first stage starts with the fertilization of the ovum and lasts until the catecholaminergic brain engine properly activates the higher levels of the brain, which then assumes the lead and integrate the different parts of the organism into a highly sophisticated entity. The first stage of the mammalian organism's development is completed when the catecholaminergic brain engine is put into gear once and for all. This is the intrauterine birth of the unique individual. The appearance of the EEG signals the transition from the first into the second stage of development.

Cells need oxygen, water, and food for life. These are first supplied, via the placenta, by the mother. The subsequent, highly complicated evolving program is devoted to ensuring independence from the mother.

The second stage of development ends with the passage of the fetus from the uterus to the outside world. From a functional point of view, birth means the transition from fetal to postnatal circulation, with the newborn infant now supplying itself with oxygen.

The third stage lasts from birth until weaning and serves to develop the skills needed for the maintenance of integrity and for the infant to supply itself with water and food.

The fourth stage lasts from weaning until the goal of goals in nature: full scale sexual maturity is reached. This is the most delightful phase of life, the glorious uphill journey. The individual progressively takes possession, on a mature level, of all abilities crucial for survival and maintenance of the species. It learns to avoid dangerous situations, masters the techniques for obtaining its food, develops procreative powers for sexual reproduction and copulates. This is, at the same time, the climax of developmental longevity.

The sexually fully mature individual fulfills its duty. Thus, to maintain the precisely balanced natural equilibrium among living organisms, the biologically "useless" individual has to be eliminated. According to the inborn program, the fifth, post-developmental stage of life (aging) begins.

The fifth stage is in essence the progressive decay of the efficiency of the catecholaminergic brain engine during the post-developmental lifespan. At some point, in an emergency situation, the integration of the parts in a highly sophisticated entity can no longer be maintained and "natural death", signaled by the disappearance of the EEG signal, sets in.

As parts of the organism remain alive, life expires with the successive dying off of different groups of cells. The outlined hypothesis suggests that quality and duration of life rests upon the inborn efficiency of the catecholaminergic brain machinery, i.e., a higher performing, longer-living individual has a more active, more slowly deteriorating catecholaminergic system

than its lower performing, shorter-living peers. To simplify this concept, I believe, that a superior brain engine allows for a better performance and a longer lifespan.

The concept clearly predicts that, as the activity of the catecholaminergic system can be improved at any time during life, it must essentially be feasible to develop a technique for transforming a lower-performing, shorter-living individual, to a better-performing, longer-living one. It therefore follows that a shift in the duration of life beyond the technical lifespan (TLS), with a still unpredictable limit, must be possible in all mammals, including the human species.

The results of the second longevity study (Knoll et al. 1994) were in harmony with this concept. In this longevity study, we started working with a random population of 28-week-old male rats and tested their sexual performance once a week. Rats that represented the two extremes in performance were selected for the study. The ones that did not display a single intromission during the four consecutive weekly-mating tests used for selection, and the ones which showed full scale sexual activity (mounting, intromission, ejaculation) in each of the four tests. Out of 1,600 sexually inexperienced 28-week-old Wistar-Logan male rats that copulated with a receptive female once a week during four consecutive weeks, 94 did not display a single intromission during the selection period, and 99 displayed at least one ejaculation in each of the four tests. The former were determined as the lowest performing (LP) rats and the latter for the highest performing (HP) ones.

After selection, we started to treat the 8-month-old rats subcutaneously with either 1 ml/kg 0.9% NaCl or with 0.25 mg/kg DEP, dissolved in 0.9% NaCl given in the same volume, three times a week, until the end of their life. Out of the 94 LP animals, 46 were treated with saline. Out of the 99 HP animals, 49 were treated with saline. The mating and learning performances of these saline-treated LP and HP rats were tested for 108 weeks. Sexual activity was tested once a week. The learning performance of the rats was tested in the shuttle box. The rats were trained once every three months for a period of five days, with 20 trials a day. In this longevity study we trained our rats in the shuttle box instead of the optimal training conditions (100 trial), only with 20 trials, to find more pronounced difference in the learning ability between high and low performing rats. Fig.2. demonstrates the highly significant difference in sexual, learning performances and life span between LP and HP rats.

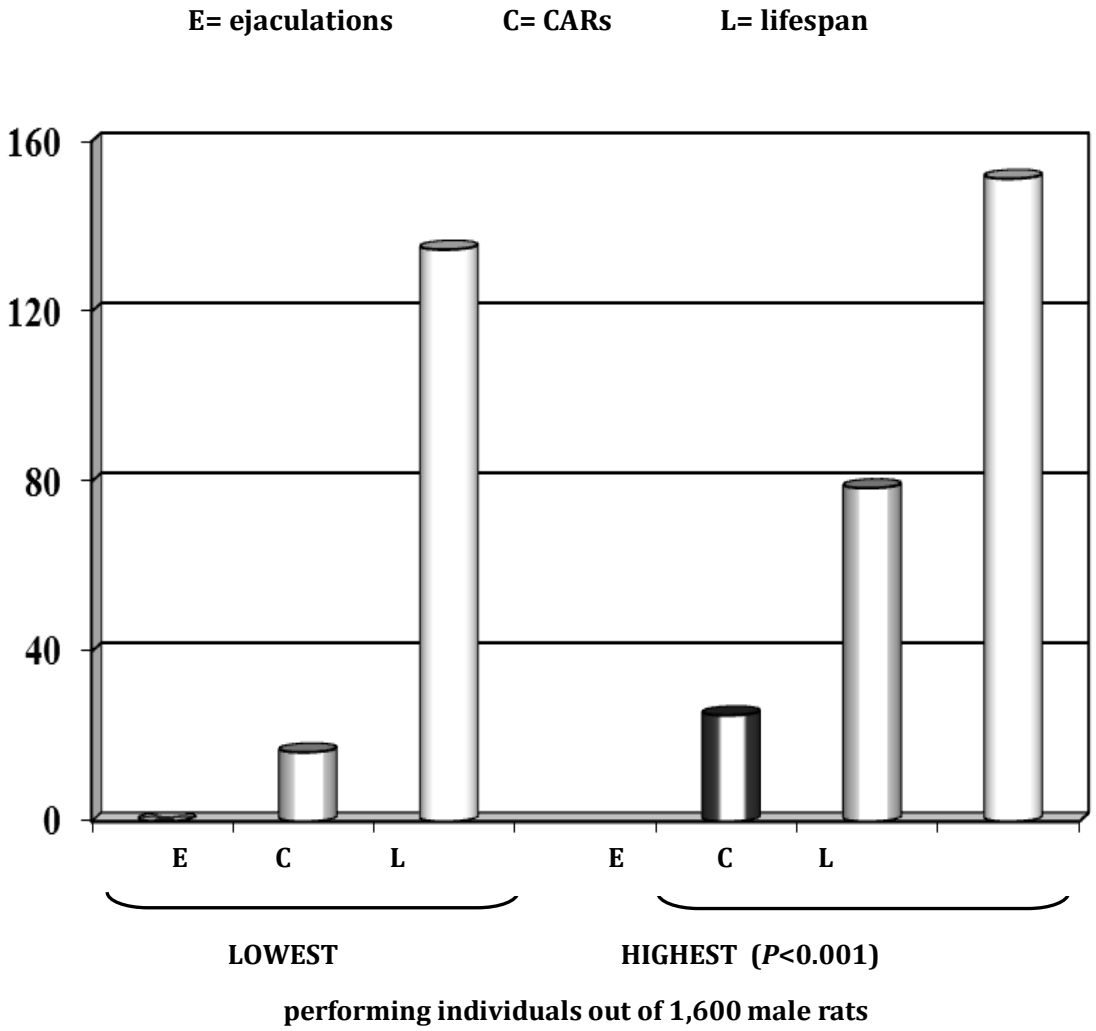


Fig.2. Illustration of the highly significant differences in sexual, learning performances and lifespan between two groups of rats selected out of 1600 28-week-old Wistar-Logan males, as the sexually lowest performing (LP) and highest performing (HP) individuals. See text for details.

The saline-treated LP rats (n=44) never displayed ejaculation during their lifetime, were extremely dull in the shuttle box and lived 134.58±2.29 weeks. The saline-treated HP rats (n=49) displayed 14.04±0.56 ejaculations during the first 36-week testing period and due to aging they produced 2.47±0.23 ejaculations between the 73-108th week of testing. They lived 151.24±1.36 weeks, significantly (P<0.001) longer than their LP peers.

Maintenance on DEP enhanced the performance of both LP and HP rats and significantly prolonged their lifespan. The DEP-treated LP rats (n=48) became sexually active as their mating performance was substantially increased and they lived 152.54±1.36 weeks which is

significantly longer than their saline-treated peers and as long as the saline-treated HP rats. The DEP-treated HP rats (n=50) were much more sexually active than their saline-treated peers. They displayed 30.04±0.85 ejaculations during the first 36-week testing period and 7.40±0.32 ejaculations between the 73-108th week of testing. Also their learning performance was substantially increased. They produced 113.98±3.23 CARs during the first 36-week-testing period and 81.68±2.14 CARs during the 73-108th week of testing. *They lived 185.30±1.96 weeks, significantly more than their saline-treated peers. Out of the 50 rats, 17 lived longer than the longest lifespan we ever observed in untreated or saline-treated Wistar-Logan rats.*

What causes the transition from developmental to post-developmental longevity?

The answer to this phenomenon began to emerge during the course of our behavioral studies on rats in the 1950s. *I consistently experienced that the hunger drive induced orienting-searching reflex activity is significantly more pronounced in younger rats than in their elderly peers* (Knoll 1957). We repeatedly corroborated this observation and last verified it in 1995 (Knoll and Miklya 1995).

Catecholaminergic neurons have a powerful activating effect on the brain. We measured the hunger-induced orienting-searching reflex activity in rats and found that *animals in a later developmental phase of life (2 months of age) were much more active than those in the early post-developmental phase (4 months of age)*. **These findings point to an enhanced catecholaminergic activity during the developmental phase of life**.

Fig.3 shows that if we measure the intensity of the orienting-searching reflex activity of hungry rats in a new surrounding as a function of time elapsed from the last feed, we observe the striking difference in activity between 2-month-old rats being in their uphill period of life and 4-month-old rats being already in their early post-developmental phase of life. We also observed the awakening of sexual drive, maturation of spermatozoa and the development of the penis in male CFY rats. From the strain we used in this experiment, it was exceptional to find copulation drive manifesting in males younger than six weeks. Although the appearance of copulation patterns usually precedes maturation of the spermatozoa and full penis development, the overwhelming majority of the males reached full-scale sexual activity by the completion of their 2nd month of life.

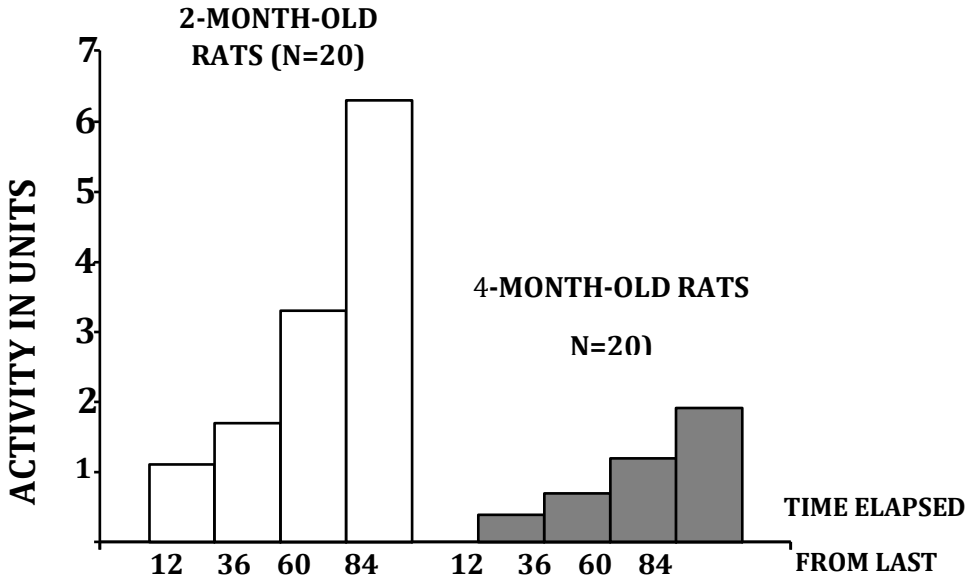


Fig.3. Intensity of orienting-searching reflex activity of hungry rats in new surroundings as a function of time elapsed from previous feed. Activity was measured and expressed in units from 0 to 10. See Knoll and Miklya (1995) for methodology and further details.

During the interval from weaning (3rd week of life) until the end of the 2nd month of age, the decisive developmental period of rats, they acquire crucial abilities for their species survival and maintenance. *The powerfully enhanced general activity in this phase strongly suggested the* ***working hypothesis*** *that a CAE mechanism operates in the catecholaminergic neurons which in service of the requirements is transformed to a high activity level during the developmental phase of life.*

1.4. Positive proof by DEP that a catecholaminergic activity enhancer (CAE) regulation works in the catecholaminergic neurons. Supportive research revealed my expectation: *the predicted enhancer regulation was detectable in the catecholaminergic neurons* (to follow the development of this research, see: Knoll 1994, 1998, 2001, 2003, 2005, 2012).

PEA and MET are highly potent releasers of catecholamine transmitters from their intraneuronal storage in the vesicles and ***this effect concealed*** *the detectability of their CAE effect (Knoll 2012). Thus, this essential mechanism remained undetected until DEP, the first PEA/MET-derivative that is devoid of the catecholamine-releasing effect of its parent compounds, enabled its discovery. The discovery of the enhancer regulation revealed that PEA and MET are primarily CAE substances.*

My first paper describing DEP as a CAE substance acting in the brain was published in

1998, and a detailed analysis was published in two reviews and a monograph (Knoll 1998, 2001, 2003, 2005).

The pharmacology and the therapeutic benefits of ®-N-methyl-N-(1-phenylpropan-2-yl)-prop-1-yn-3-amine [Selegiline, (-)-Deprenyl, Eldepryl, Jumex, Zelepar, Emsam, Anipryl, and more than 100 further trade names] were described in thousands of papers, dozens of reviews and a couple of books. The history about the development of DEP as the first selective inhibitor of B-type MAO and the first CAE substance was recently published in the Archives of the International Network for the History of Neuropsychopharmacology (INHN) (Miklya 2014a; inhn.org. Archives, Miklya Collection, March 13, 2014).

DEP was created in the 1960s, in the midst of the golden era when within less than twenty years the development of crucially new families of important pharmacological agents, MAO-inhibitors, phenothiazines, tricyclic antidepressants and uptake inhibitors, were developed. These discoveries led to the birth of neuropsychopharmacological science. It revolutionized the principles of behavioral studies and radically altered human attitudes toward derangements in psychic function. A volume edited in 1998 by Ban et al., a retrospective of "The Rise of Psychopharmacology and the Story of CINP", stated that "Deprenyl, the first catecholaminergic activity enhancer" (pp. 91-94) belongs to "The Mainstream of Drug Development."

As previously mentioned, my behavioral studies compelled me to begin in the early 1960s the SAR-study which led to the development of DEP, a PEA/MET-derivative with a peculiar pharmacological profile. The first paper was published in 1964 in Hungarian (Knoll et al. 1964) and in 1965 in English (Knoll et al. 1965). We used the racemic compound under the code name E-250 in the first series of experiments. The (-) enantiomer of E-250 (later named (-)-deprenyl/selegiline) was selected for detailed pharmacological analysis and finally for therapeutic use.

AMPH and MET (first synthesized in 1887, resynthesized in 1929 and used from the early 1930s as drugs) are synthetic PEA-derivatives. PEA, a potent releaser of the catecholamine transmitters from the vesicles, the natural sympathomimetic trace amine with a rapid oxidative deamination by MAO, is short acting. AMPH and MET, not metabolized by MAO, even slight inhibitors of this enzyme, are the long acting PEA-derivatives.

Out of a series of newly synthesized patentable MET-derivatives, I selected E-250 (later named (-)-deprenyl) as the most promising for our planned behavioral studies. I also saw the potential of this substance as a new spectrum antidepressant.

Unlike newly synthetized derivatives that increased blood pressure, like its parent compound MET, *E-250 was an exception, since it lowered blood pressure and inhibited AMPH-induced vasopressor effect* (Knoll et al. 1965; Fig. 1). From a therapeutic point of view, this finding was a promising beneficial change. Further studies soon revealed that the development of a compound with the profile of DEP was quite exciting.

In 1963, a calamitous number of clinical reports (Womack, Foster, Maan, Davies) appeared in The Lancet concerning patients treated with MAO inhibitors (tranylcypromine, nialamide, pargyline) who developed temporary clinical symptoms (hypertension, palpitation, neck stiffness, headache, nausea, vomiting), similar to a paroxysm produced by pheochromocytoma. Blackwell correctly observed that these hypertensive crises are associated with the ingestion of high amounts of tyramine in cheese, and MAO inhibitors impede metabolism (a.k.a. the "cheese effect") (Blackwell 1963). Cheese and many other foods containing tyramine provoke hypertensive episodes in patients treated with MAO inhibitors. Because of the "cheese effect", this important, novel group of therapeutic agents was restricted from clinical use despite its growing popularity.

In contrast, DEP is an exceptional MET-derivative not only devoid of the vasopressor effect of amphetamines but also an inhibitor of the pressor effect of tyramine. Thus, DEP is devoid of the "cheese effect".

As expected, studies revealed that in specified dose-range which blocks selectively MAO-B activity, *DEP, in contrast to the known MAO inhibitors, did not potentiate the effect of tyramine but inhibited it.* This effect of deprenyl was first demonstrated in a study with cats and isolated vas deferens of rats. The special therapeutic value of DEP as a peculiar tyramine inhibitor and potent MAO inhibitor was discussed and summarized in a paper published in 1968 (Knoll et al. 1968).

In the same year we published the unique behavior of DEP, Johnston described a substance, later named clorgyline, which would later become a world-wide experimental tool in MAO research (Johnston 1968). He primarily realized that clorgyline preferentially inhibits the deamination of serotonin, and this important finding was soon confirmed by Hall et al. (1969).

Johnston proposed the existence of two forms of MAO, "type A" and "type B". The former is selectively inhibited by clorgyline, and the latter is relatively insensitive to it. Johnston's nomenclature has become widely accepted and is still in use. Clorgyline remained the classic selective inhibitor of A-type MAO.

For further studies, a selective inhibitor of MAO-B was strongly needed. I was astute enough to realize that DEP was the missing, highly selective inhibitor of MAO-B and presented this finding in my lecture at the First International MAO Meeting in Cagliari (Sardinia) in 1971. DEP was used, thereafter, as the specific experimental tool to analyze B-type MAO. The first paper which described this novel property (Knoll and Magyar 1972) has become a citation classic ten years later (Knoll J, This Week's Citation Classic, January 15, 1982). DEP's selective MAO-B inhibitory effect was at the center of our interest for many years. However, it delayed the discovery of the drug's enhancer effect. The MAO inhibitory effect of the compound led to the first clinical application of DEP.

Because of the serious side effects of levodopa in Parkinson's disease, Birkmayer and Hornykiewicz attempted to achieve a levodopa-sparing effect with the concurrent administration of levodopa with a MAO inhibitor. As such combinations frequently elicited hypertensive attacks; they soon terminated this line of clinical research (Birkmayer and Hornykiewicz 1962).

Unfortunately, 1960s Hungary was isolated from the western world's mainstream science, we published our papers in the Acta Physiologica Hungarica, and our results remained largely unnoticed. Since our studies confirmed that E-250 is antagonizing tyramine's effect, I asked my close friend and classmate, Ervin Varga, who worked as psychiatrist in our University Clinic, to test the antidepressant effect of E-250 and confirm the lack of the "cheese effect" in a preliminary trial.

In 1965 Varga published a preliminary note (in German) on the promising results of the running clinical trial with racemic E-250 on depressed patients (Varga 1965), and he wrote with his coworker the first paper, in English, describing that racemic E-250 is an efficient, prompt acting antidepressant (Varga and Tringer 1967). In 1971 they wrote the first paper demonstrating that (-)-E-250, now known as (-)-deprenyl/ selegiline, is a potent antidepressant (Tringer and Varga 1971). In retrospect it is incredible that selegiline was only first registered as an antidepressant in 2006 in the United States (Emsam), though our first paper on E-250 which proposed this indication appeared in the Hungarian version in 1964 and in the English version in 1965 (Knoll et al. 1964, 1965).

Varga also found that E-250 is free of the "cheese effect" in humans. As stated in personal communication, he said "Even provocative cheese consumption failed to produce headache or hypertensive crisis" (Knoll et al. 1968; p.111). Varga moved to the USA, where he still lives, and he discontinued his clinical studies with selegiline. His convincing preliminary study which confirmed that E-250 is devoid of the "cheese effect" was never completed and

has remained unpublished. It marks the era in Hungary in the 1960s that in the discussion of the Knoll et al. 1968 paper also two other Hungarian studies are mentioned which confirmed that E-250 was devoid of the "cheese effect" (Kardos and Füredi 1966; Juhász personal communication). None of them were completed, but later performed studies confirming these observations.

My proposal that DEP is free of the "cheese effect" was tested by Sandler and his co-workers in London with volunteers, and the results were published in two convincing papers in 1978. They confirmed that DEP is an MAO inhibitor free of the "cheese effect" which aligned with our findings in animal experiments. After pretreatment with DEP parkinsonian volunteers who had received levodopa or levodopa+carbidopa suffered no adverse pressor reaction after challenged with oral tyramine in considerably greater amounts the dose likely to be encountered in a normal diet (Elsworth et al. 1978; Sandler et al. 1978).

Considering the peculiar pharmacological profile of DEP, Birkmayer in Vienna was the first clinician who dared to combine DEP with levodopa in Parkinson's disease. The trial was successful. The levodopa-sparing effect was achieved in patients without signs of significant hypertensive reactions (Birkmayer et al. 1977). This study initiated and a following Lancet Editorial (September 25, 1982) enhanced the world-wide use of DEP in Parkinson's disease. DEP achieved its place in research and therapy as the first selective inhibitor of MAO-B. Prior to the discovery of the CAE effect of DEP, it was my firm belief that the selective inhibition of B-type MAO is responsible for the drug's beneficial therapeutic effects. In my lecture at the 'Strategy of Drug Research' IUPAC/IUPHAR Symposium in Noordwijkerhout (The Netherlands) in 1982, I presented experimental evidence that preventive daily administration of DEP during the post-developmental phase of life is an unexpected chance to improve the quality and prolong the duration of mammalian life (Knoll 1982).

Robinson et al. were the first to show that MAO activity progressively increases in the aging brain (Robinson et al. 1971). In both human (Fowler et al. 1980) and rat brains (Mantle et al. 1976; Strolin Benedetti et al. 1980) B-type MAO activity only increases over time. The age-dependent change in MAO activity is entirely due to an increased cerebral enzyme concentration (Fowler et al. 1980). Over time, MAO-B activity is selectively enhanced, so it is reasonable to conclude that this change is mainly responsible for the aging-related decay in the efficiency of the catecholaminergic brain engine. Thus, I presented the following working hypothesis:

"In the aging brain, there is a loss of neurons, compensated for by a proliferation of glial cells. We might thus predict that dopaminergic transmission and 'trace-aminergic' modulation

in the brain declines in senescence because of the loss of neurons and because of the increased monoamine oxidase (MAO)-B activity present in the glia. The hypothesis was forwarded that the significant increase in the incidence of depression in the elderly, the age-dependent decline in the male sexual vigor and the frequent appearance of parkinsonian symptoms in the latter decades of life might be attributed to a decrease of dopamine and 'trace amines' in the brain. The possibility to counteract these biochemical lesions of aging by chronic administration of DEP, a selective inhibitor of MAO-B, which facilitates dopaminergic and 'trace-aminergic' activity in the brain and is a safe drug in man, was proposed. The restitution and long term maintenance of full scale sexual activity in aged male rats continuously treated with DEP was demonstrated as an experimental model in support of the hypothesis that the long term administration of small doses of DEP may improve the quality of life in senescence" (Knoll 1982).

The high pressure liquid chromatography (HPLC) method with electrochemical detection allows exact measurement of the continuously emitted catecholamines from freshly excised brain tissue. This method ensured us to obtain unequivocal experimental evidence regarding the operation of the enhancer regulation in the life-important catecholaminergic and serotonergic systems of the brain stem. In 1993, we began to use this technique to measure the amount of DA emitted from the striatum, substantia nigra, and tuberculum olfactorium, as well as NE from the locus coeruleus and SER from the raphe.

In 1994 we presented the results from the first series of experiments performed with the HPLC method which demonstrated that multiple, small dose administration of DEP keeps the catecholaminergic and serotonergic neurons on a significantly higher activity level, and how ***DEP***'s ***peculiar enhancer effect is unrelated to MAO-B inhibition*** (Knoll and Miklya 1994).

Table 1 and 2 furnish data to illustrate the essential findings from this first series of experiments. In these experiments, the rats were daily treated for 21 days subcutaneously with DEP, PPAP and MET, respectively, and the amount of catecholamines and SER emitted from the isolated, discrete brain's regions (DA from the striatum, substantia nigra and tuberculum olfactorium; NE from the locus coeruleus and SER from the raphe) was measured by HPLC method. We also measured the dose-related effects of DEP, PPAP and MET.

The brain samples were isolated 24 hours after the last drug injection, *thus providing an exact measurement of the daily administration of the enhancer substances for 21 days*.

We investigated MET's effect on the catecholaminergic and serotonergic neurons because it is the parent compound of DEP. We tested PPAP as the DEP-analog containing a

propyl-group which is unable to make a covalent binding with the flavin in MAO-B rather than the propargyl-group in DEP. Thus, PPAP leaves MAO-B activity unchanged (Knoll et al. 1992). The data in Table 1 demonstrates that each of the tested compounds possess a dopaminergic activity enhancer (DAE) effect.

Table 1

		Amount of dopamine (nmoles/g tissue) emitted from the brain sample within 20 min		
Treatment	**Daily dose (mg/kg)**	**Striatum**	**Substantia nigra**	**Tuberculum olfactorium**
MALE				
Saline	0, 3 ml/100g	2.72 ± 0.10	2.96 ± 0.13	2.58 ± 0.06
DEP	0.01	3.27 ± 0.14***	4.17 ± 0.31****	3.08 ± 0.18***
	0.05	4.42 ± 0.09****	4.47 ± 0.09****	4.53 ± 0.33****
PPAP	0.10	5,17 ± 0.29****	4.52 ± 0.46****	5.02 ± 0.21***
MET	0.05	3.77 ± 0.08****	3.53 ± 0.23*	3.65 ± 0.19****
FEMALE				
Saline	0, 3 ml/100g	2.42 ± 0.06	2.80 ± 0.03	2.42 ± 0.1
DEP	0.01	3.20 ± 0.08***	3.18 ± 0.07***	3.37 ± 0.09***
	0.05	3.73 ± 0.27***	3.55 ± 0.19***	3.35 ± 0.18***
PPAP	0.10	4.90 ± 0.26****	5.65 ± 0.26****	5.02 ± 0.21****
MET	0.05	2.87± 0.14***	3.62 ± 0.10****	2.85 ± 0.11**

*Values are means ± S.E.M.; n=12; *p<0.05; **p<0.02; ***p<0.01; ****p<0.001.*

Table 1. Proof of the dopaminergic activity enhancer (DAE) effect of (-)-deprenyl (DEP), (-)-PPAP (PPAP) and (-)-methamphetamine (MET). Experimental evidence that male and female rats treated for 21 days, subcutaneously, with a single low daily dose of a catecholaminergic activity enhancer (CAE) substance keeps the dopaminergic neurons on a significantly enhanced activity level even 24 hours after the last injection.

Table 2

		Amount of norepinephrine and serotonin (nmoles/g tissue) emitted from discrete brain samples within 20 min	
Treatment	**Daily dose (mg/kg)**	**Locus coeruleus**	**Raphe**
MALE			
Saline	0.3 ml/100g	4.10 ± 0.18	0.410 ± 0.18
DEP	0.01	6.13 ± 0.62***	0.519 ± 0.05
	0.05	7.88 ± 0.23****	0.357 ± 0.04 **
PPAP	0.10	7.93± 0.69****	0.466 ± 0.03
MET	0.05	6.20 ± 0.26 ****	0.214 ± 0.002****
FEMALE			
Saline	0.3 ml/100g	3.67 ± 0.23	0.460 ± 0.03
DEP	0.01	3.97 ± 0.23	0.444 ± 0.02
	0.05	7.70 ± 0.10***	0.370 ± 0.04
PPAP	0.10	7.93 ± 0.69****	0.390 ± 0.01
MET	0.05	6.20 ± 0.26****	0.300 ± 0.04**

*Values are means ± S.E.M.; n=12; *p<0.05; **p<0.02; ***p<0.01; ****p<0.001.*

Table 2. Proof of the noradrenergic activity enhancer (NAE) effect of (-)-deprenyl, (-)-PPAP and (-)-methamphetamine (MET). Experimental evidence that male and female rats treated for 21 days, subcutaneously, with a single low daily dose of a catecholaminergic activity enhancer (CAE) substance keeps the noradrenergic neurons on a significantly enhanced activity level even 24 hours after the last injection. In contrast, the CAE substances did not enhance the activity of the serotonergic neurons.

Table 2 shows that both male and female rats treated with DEP, PPAP or MET had

significantly enhanced activity in their noradrenergic neurons. In contrast, the tested compounds are devoid of a serotonin activity enhancer (SAE) effect. There is a significant decrease in the activity of the serotonergic neurons in male rats treated with 0.05 mg/kg DEP or MET, and in females treated with 0.05 mg/kg MET. *The data clearly demonstrates that the maintenance of both male and female rats on a small, daily dose of an enhancer substance maintains the catecholaminergic neurons on a significantly higher activity level.*

The fact that PPAP is a potent enhancer of catecholaminergic neuronal activity furnishes direct evidence that DEP's CAE effect is unrelated to MAO-B inhibition. Moreover, rat cerebral MAO-B is blocked by 0.15 mg/kg DEP to 50% and 0.25 mg/kg is the dose to fully inhibit MAO-B activity. This means that a 0.01-0.05 mg/kg daily dose of DEP is completely effective in enhancing catecholaminergic neuronal activity, and it leaves the activity of MAO-B in the brain practically unchanged. Since repeated administration of DEP allows for an unchanged MAO inhibition pattern (Ekstedt et al. 1979), there is no doubt that the enhanced catecholaminergic activity in the brain following multiple, small dose administration of DEP is unrelated to MAO-B inhibition.

DEP, PPAP and MET are primarily CAE substances. PEA, their original parent compound, is a trace amine in the mammalian brain known to release catecholaminergic transmitters from their storage places. It is primarily a natural CAE substance. MET, the synthetic PEA-analog with a long lasting effect, shares a similar pharmacological spectrum with PEA. In contrast, DEP and PPAP are the first PEA-derivatives unable to release catecholamines from their stores. As elaborated upon in this paper, further studies confirmed that PEA is primarily a natural CAE substance present in mammalian brains and releases catecholamine transmitters from their storage places only in high concentrations (Knoll et al. 1996b).

Fig.4. shows the chemical structure and differences in the pharmacological profile between PEA, MET, DEP and PPAP. It is of practically importance that the CAE effect is long lasting. The enhanced catecholaminergic activity in rats treated for 21 days with a single, low daily dose of a CAE substance was measured 24 hours after the last dose.

Figure 4

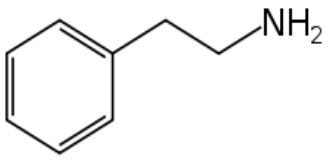
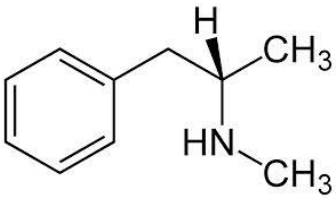
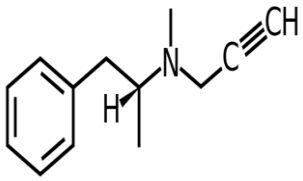
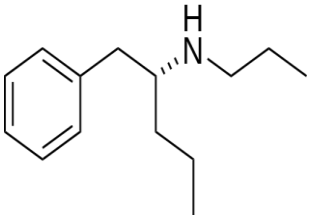
COMPOUND	CHEMICAL STRUCTURE	RELEASER OF CATECHOL-AMINES	SELECTIVE INHIBITOR OF MAO-B	CATECHOLAMINERGIC ACTIVITY ENHANCER
β-PHENYL-ETHYLAMINE **[PEA]**		+	-	+
(-)-METHAMPHET-AMINE **[MET]**		+	-	+
(-)-DEPRENYL/ SELEGILINE **[DEP]**		-	+	+
(-)-1-PHENYL-2-PROPYLAMINO-PENTANE **[PPAP]**		-	-	+

Fig. 4. Chemical structure and differences in the pharmacological profile between PEA, MET, DEP and PPAP.

1.5. Confirmation that β-phenylethylamine (PEA), the parent compound of methamphetamine (MET) and DEP, is primarily a native CAE substance and in high concentrations only a releaser of catecholamines from their intraneuronal

pools. Fischer et al. discovered that PEA is an endogenous trace amine in the brain and this finding was soon corroborated (Fischer et al. 1972; Saavedra 1974; Willner et al. 1974). Fischer et al. claimed that urinary excretion of free PEA is reduced in depressed patients, thus suggesting the hypothesis that a PEA-deficit is one of the biochemical lesions of depression (Fischer et al. 1968, 1972). Sabelli and his coworkers hypothesized that PEA might play a role in affective behavior (Sabelli and Giardina 1973; Sabelli and Mosnaim 1974). Since Borowsky and his team identified a family of mammalian G-protein-coupled receptors and found that these receptors are activated by PEA and tryptophan (Borowsky et al. 2001), they inadvertently detected a family of enhancer receptors (Knoll 2005).

Because of PEA's effects it was and has remained common knowledge that the trace amine is an indirectly acting sympathomimetic agent which displaces catecholaminergic transmitters from their storage sites. The enhancer regulation's discovery revealed that *PEA is a CAE substance in low concentrations* and releases catecholamines from their storage sites in higher concentrations (Knoll et al. 1996b). *The releasing effect of PEA is detectable on isolated organ preparations with catecholaminergic innervation. As a CAE substance, PEA acts in the brain (Knoll 1998) and is usually peripherally undetectable (Knoll et al. 1996b).*

A ***capacitance*** *vessel preparation, the helical strip of the pulmonary artery of the rabbit*, is particularly well suited to measure the displacement of NE molecules from their storage sites by PEA, and the CAE effect is undetectable. Fig.5 shows that the cumulative administration of 1.4 μg/ml PEA in three consecutive small doses (0.2, 0.4, and 0.8 μg/ml) neither increased stimulation induced contractions (lack of CAE effect), nor smooth muscle tone (lack of release of NE). Following an additional 6 μg/ml PEA there was a very slight increase in the smooth muscle tone in resting state. However, there was no significant change in the stimulation-induced contractions. After administering 10, 20 and 40 μg/ml PEA, there was a dose-related increase in the smooth muscle tone in the resting state due to the stoichiometric displacement of NE by PEA, but there was no sign of a CAE effect. The electric stimulation induced contractions remarkably decreased when the highest concentration was given.

Figure 5

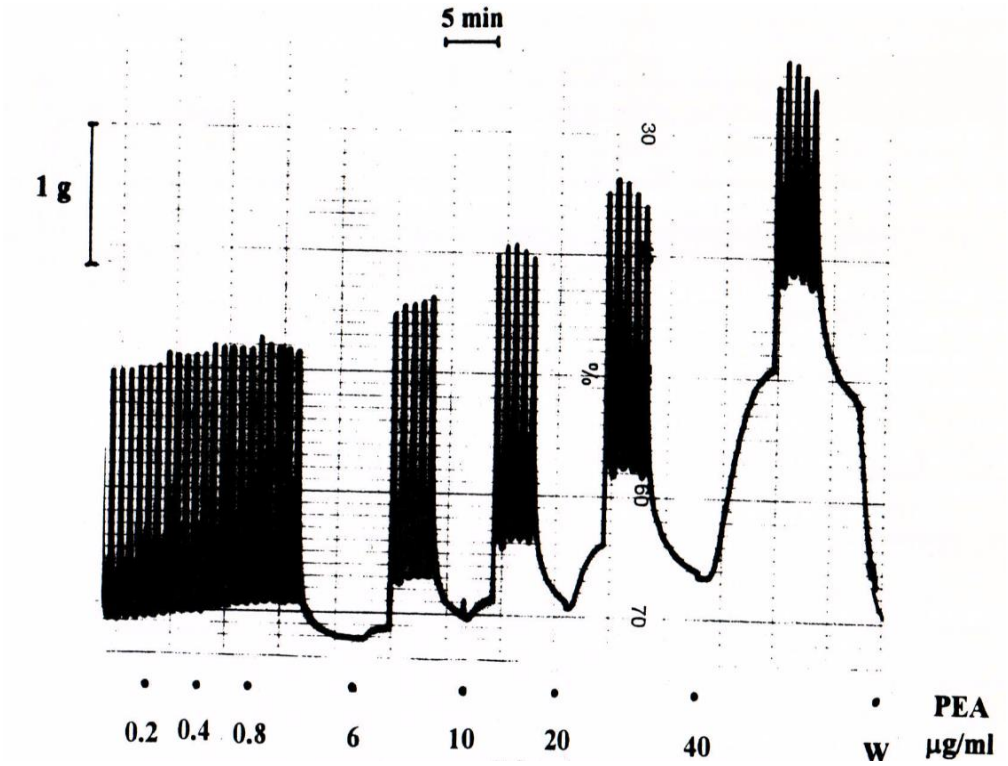


Fig.5. Pulmonary artery strip from a rabbit. Note the PEA-induced dose-related gradual increase in smooth muscle tone (due to displacement of NE) and the absence of enhanced response to electric stimulation in the presence of PEA (lack of CAE effect). Stimulation: 3Hz, 12 pulses; W=washing

DMI (5 µg/ml) completely prevents the NE displacing effect of PEA by inhibiting the neuronal transport of this amine. Also, PPAP, the PEA-derivative with a potent CAE activity but devoid of NE displacing property, is an efficient inhibitor of PEA's effect (ID_{50} = 1.22 x 10^{-6} M) (Knoll et al. 1992). The antagonism is due to competition for the neuronal transport system.

The *rabbit's perfused ear artery, a* ***resistance*** *artery preparation*, is an exceptional sample of a vascular smooth muscle suitable for investigating the dual effect of PEA on catecholaminergic transmission outside of the brain (Knoll et al. 1996a).

There are two mechanisms which lead to the emission of high amounts of NE from the end organs in the preparation: NE's release via the exocytosis of vesicles in response to electric stimulation and NE's continuous outflow via the transmitter's displacement by an indirectly acting sympathomimetic agent, like for example PEA, which has to be measured in the resting state when exocytosis of vesicles does not occur.

Fig.6 shows the dual effect of PEA on the perfused central ear artery of a rabbit.

Part A in Fig.6 shows the dose-related CAE effect of PEA in a concentration range (0.2-0.8 μg/ml) which unchanged smooth muscle tone. Much higher concentrations of PEA 4-6 μg/ml were needed for displacing NE (Part B). DMI (5 μg/ml) prevented PEA-induced displacement of NE (Part C), whereas the CAE effect was still detectable. Note the enhancement of stimulation induced smooth muscle contractions in Part C. Part D shows the CAE effect of small amounts of PEA in the presence of DMI.

Figure 6

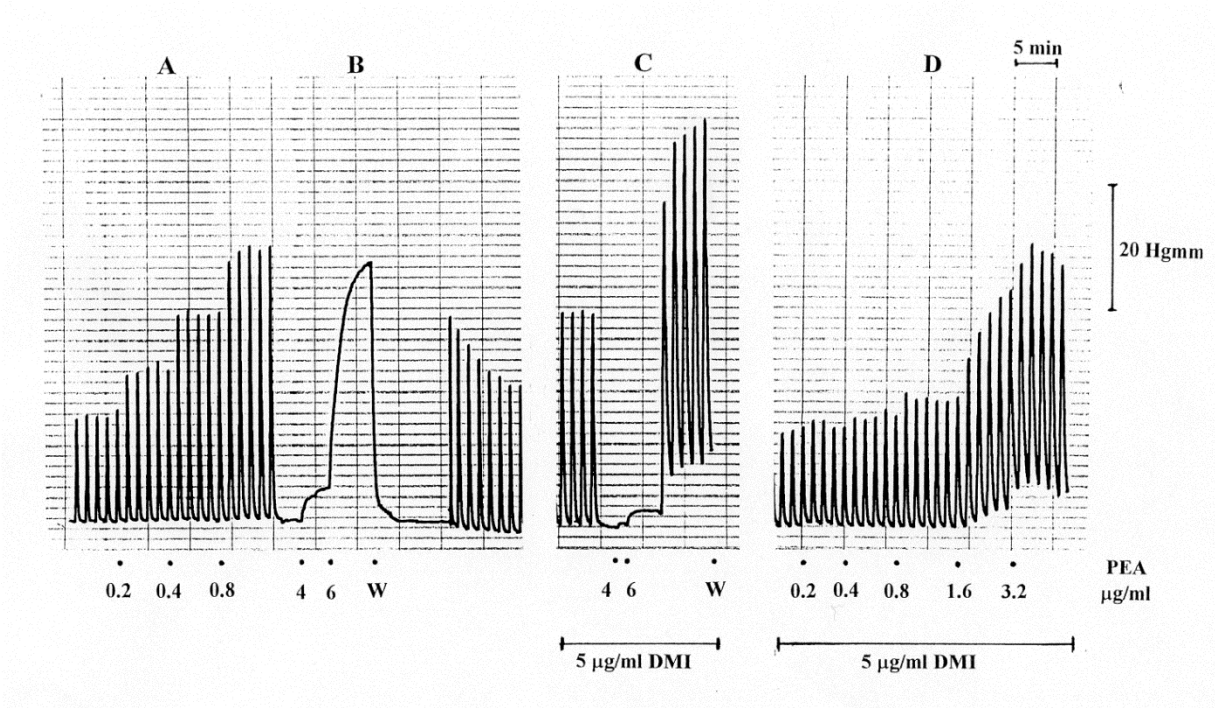


Fig.6. The rabbit's-perfused-central ear artery. Part A: the PEA-induced dose-related enhancement of the response to electric stimulation (CAE effect); Part B: increased smooth muscle tone in the resting state following the administration of PEA (displacement of NE); Part C: the inhibition of latter effect in the presence of DMI; Part D: the ineffectiveness of DMI to block the CAE effect of PEA.
Stimulation: 3Hz, 9 pulses; W= washing.

We selected the isolated rat's brain stem to measure sympathomimetic amines' CAE effect (Knoll et al. 1996b). Fig.7. shows PEA's CAE effect on an isolated rat brain stem and the ineffectiveness of DMI to block the CAE effect.

AMPH and MET, the PEA-derivatives not metabolized by MAO, are like PEA CAE substances, and in substantially higher concentrations they release catecholamines from their storage sites. The releasing effect of PEA, AMPH and MET concealed their CAE effect which remained undetected. DEP's development, the first PEA-derivative devoid of the catecholamine releasing property made the detection of PEA and amphetamines' CAE effect possible.

Figure 7

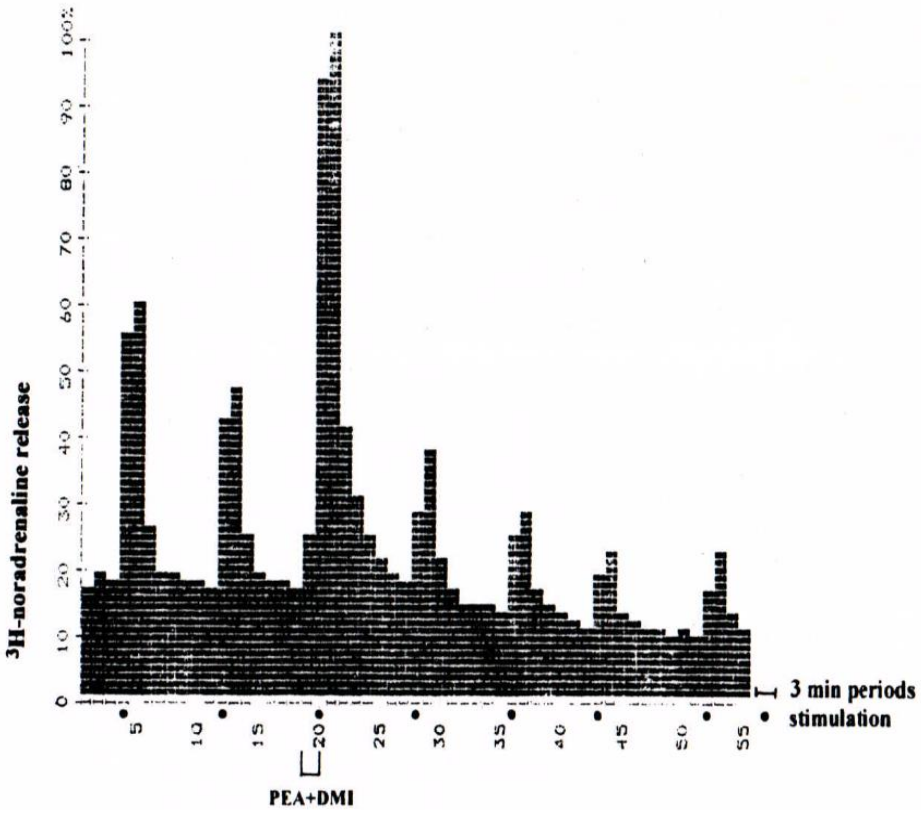


Fig.7. The enhanced release of [^{3}H]-NE (CAE effect) from an isolated rat brain stem in the presence of 10 □g/ml PEA + 5□g/ml DMI.

Figure 8

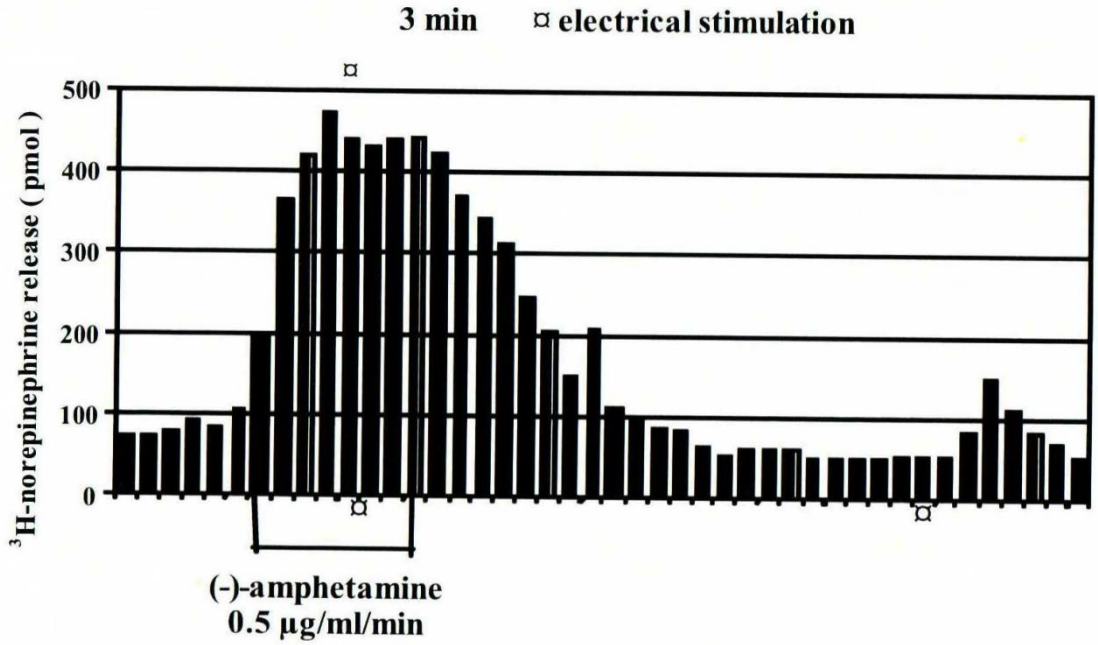


Fig.8. Demonstration that (-)-amphetamine is inducing a long-lasting spontaneous release of [^{3}H]-norepinephrine from an isolated rat brain stem. Electrical stimulation of the organ at the peak of the norepinephrine-releasing effect is ineffective, thus the enhancer effect is undetectable.

Fig.8. illustrates that AMPH-induced release of [^{3}H]-NE from the stores completely conceals the detectability of the CAE effect.

1.6. Primary evidence for the continuous tense excitement in the enhancer-sensitive catecholaminergic and serotonergic neurons at the developmental (uphill) period of life, from weaning until sexual maturity. During the interval from weaning (3rd week of life) until the end of the 2nd month of age animals acquire crucial abilities for species survival and maintenance. Based on the observation, shown in Fig.3, that 2-month-old famished rats are significantly more active than their 4-month-old peers, we checked their dopaminergic, noradrenergic and serotonergic activities in the brain before weaning (in 2-week-old rats); during the crucial developmental phase, from weaning to sexual maturity (in 4- and 8-week-old rats); and in the early post-developmental phase of life (in 16- and 32-week-old rats). As an indicator of the basic activity of catecholaminergic and serotonergic neurons in the brain, we measured the amount DA of emitted from the striatum, substantia nigra and tuberculum olfactorium,

NE from the locus coeruleus, and SER from the raphe, within a 20 min period, in male and female rats (Knoll and Miklya 1995).

We found that from weaning until the 2nd month of life, the rat's dopaminergic system was significantly more active than either before or after that period.

Fig.9 demonstrates a dramatic increase in the release of DA from the striatum and tuberculum olfactorium after weaning (4th week) and the return of DA's release to the pre-weaning level (2nd week) in sexually mature rats (32nd week). This explains why, as demonstrated in Fig.3, food-deprived rats in their developmental phase of life were significantly more mobile in an open field than their peers already in their early post-developmental phase of life. Our finding regarding the age-related changes in the dopaminergic tone in the rat brain of Wistar rats was confirmed on Long Evans Cinnamon rats (Samuele et al. 2005; Table 4).

Figure 9

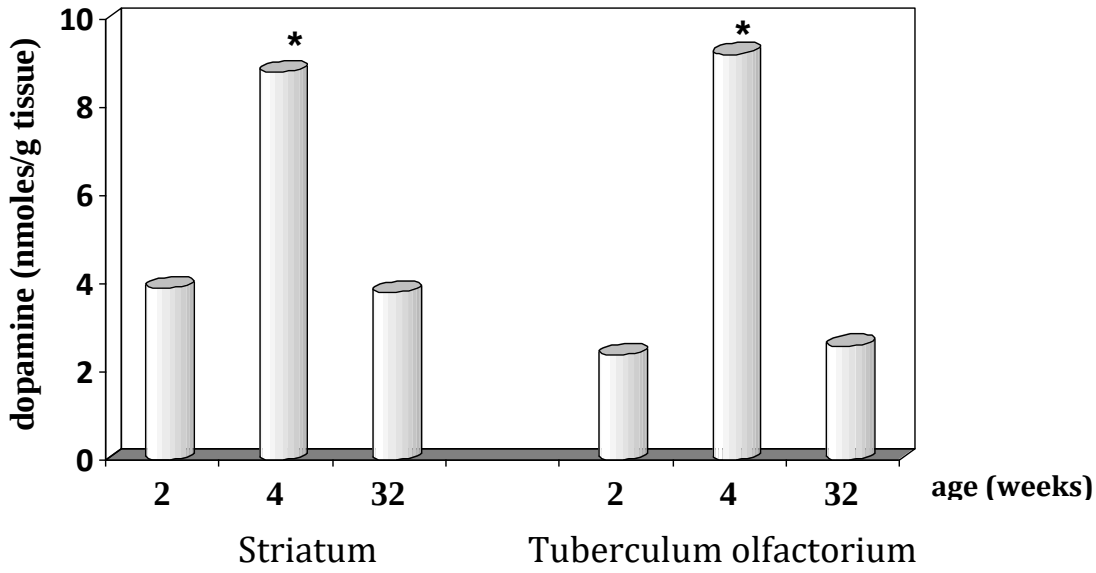
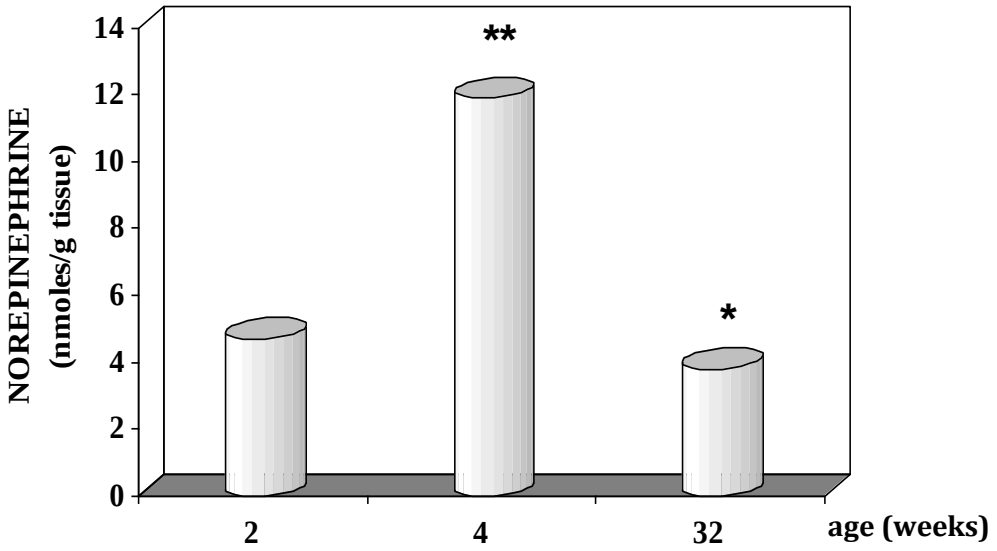
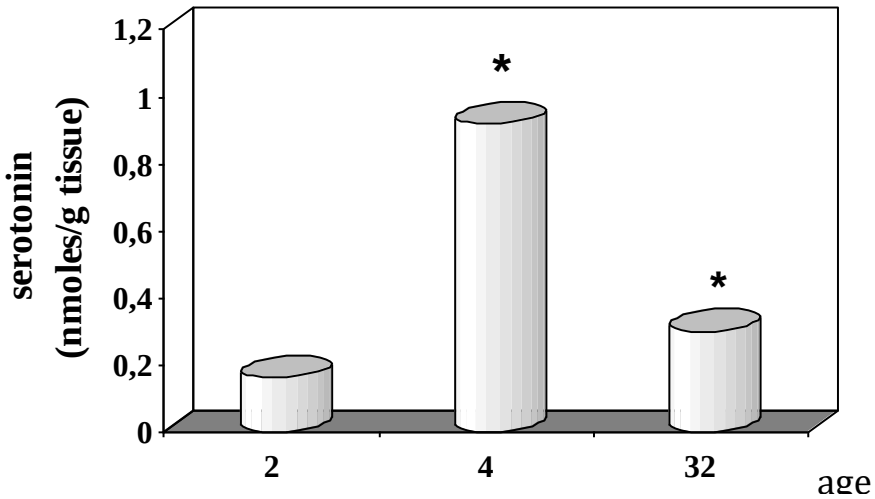


Fig.9. Release of dopamine from the striatum and tuberculum olfactorium, respectively, of male rats belonging to different age cohorts. N=12; * $P<0.001$. For details see Knoll and Miklya (1995).



L O C U S C O E R U L E U S

Fig.10. Release of norepinephrine from the locus coeruleus of male rats belonging to different age cohorts. N=12; * *P<0.01*, ***P* <0.001. For details see Knoll and Miklya (1995)



R A P H E

Fig.11. Release of serotonin from the raphe of male rats belonging to different age cohorts. N=12; * *P<0.01* **P <0.001. For details see Knoll and Miklya (1995).

The release of NE from the locus coeruleus (Fig.10) and the release of SER from the raphe (Fig.11) show the same powerful increase after weaning and the return to the pre-weaning level in sexually mature rats as DA (for details see Knoll and Miklya, 1995).

In summary, we found that the enhancer regulation starts working on a higher activity level after weaning, and this state of enhanced activity continues in existence until the completion of full scale sexual development, with a rapid rate of decay thereafter. It is obvious that as soon as sexual maturity was reached, the catecholaminergic tone changes from a "hyperactive" to an "economy" state, signaling the transition from a developmental to a post-developmental (aging) phase of life. We may also conclude that a more intensive enhancer regulation between weaning and sexual maturity is responsible for the exuberant physical strength and mental vigor of mammals in their uphill period of life.

1.7. How sexual hormones return the significantly enhanced catecholaminergic/serotonergic activity, characteristic to the uphill period of life, to the pre-weaning level, thus terminating the developmental phase of life. Since we measured in both male and female rats a significantly more pronounced enhancer regulation in the dopaminergic, noradrenergic and serotonergic neurons from the discontinuation of breast feeding (end of the 3rd week of age) until the appearance of sexual hormones (end of the 2nd month of life), we deduced that sexual hormones play the key role in terminating the developmental phase of life (Knoll et al. 2000).

The regulation of sexual hormones starts working in the rat with full capacity only at the end of the 2nd month of age. This rapid decrease in NE, DA and SER from selected discrete brain regions appeared synchronously with the completion of sexual maturity. Thus, it was reasonable to assume that sexual hormones dampen the enhancer regulation in the catecholaminergic and serotonergic brain stem neurons, and this is the mechanism which terminates developmental longevity as well.

In order to qualify these observations we castrated three-week-old male and

female rats and measured the release of catecholamines and SER from selected discrete brain regions at the end of the third month of their life. We found that in male rats the amount of catecholamines and SER released from the neurons was significantly higher in castrated than in untreated or sham operated rats, signaling that sex hormones inhibit enhancer regulation in the brain (Table 3).

To further analyze this effect of sex hormones, we treated 4-week-old male and female rats subcutaneously with oil (0.1 ml/rat), testosterone (0.1 mg/rat), estrone (0.01 mg/rat) and progesterone (0.5 mg/rat), respectively, and measured their effect on the enhancer regulation. Twenty-four hours after a single injection with the hormones, the release of NE, DA and SER was significantly inhibited in the testosterone-, or estrone-treated rats (Table 4), but remained unchanged after progesterone treatment (Table 5). In rats treated with a single hormone injection, testosterone in the male and estrone in the female was the significantly more effective inhibitor. Remarkably, the reverse order of potency was found in rats treated with daily hormone injections for 7 or 14 days (Table 6 and 7). After a two-week treatment with the hormones, estrone was found in the male and testosterone in the female as the significantly more potent inhibitor of the enhancer regulation.

The data prove that sex hormones terminate the hyperactive phase of life by dampening enhancer regulation in the catecholaminergic and serotonergic neurons. They initiate the transition from the developmental phase of life to post-developmental longevity, from adolescence to adulthood. Meanwhile, this change is also the beginning of the slow, continuous decay of the enhancer regulation in catecholaminergic and serotonergic neurons in the brain stem. Consequentially, the fixation of inextinguishable conditioned reflexes (ICRs) and the acquisition of drives are subject to an irresistible, slowly progressing, age-related decline until death.

Although the individual variation in decline of behavioral performances with passing time is substantial, the process occurs in every brain. Both the decay in brain performances as well as the potential for the manifestation of aging-related neurodegenerative diseases (Parkinson's, Alzheimer's) increases with the physiologically irrepressible aging of the brain. *It is obvious that only the development of a safe and efficient preventive pharmacological intervention, starting immediately after the completion of sexual maturity, can significantly slow brain aging.*

Table 3

	Amount of biogenic amine (nmoles/g tissue) emitted from the tissue within 20 min				
	Dopamine			**Norepinephrine**	**Serotonin**
MALES	**Striatum**	**Substantia nigra**	**Tuberculum olfactorium**	**Locus coeruleus**	**Raphe**
Untreated	3.4±0.008	4.8±0.17	3.5±0.15	3.9±0.12	0.334±0.01
Sham operated	3.3±0.11	5.2±0.34	3.5±0.16	3.9±0.09	0.329±0.02
Castrated	4.4±0.17**	7.4±0.21**	4.7±0.12**	5.5±0.22**	0.921±0.02**
FEMALES					
Untreated	3.0±0.14	4.5±0.14	2.9±0.05	3.1±0.07	0.337±0.01
Sham operated	2.9±0.13	4.3±0.17	2.8±0.18	3.0±0.05	0.339±0.01
Castrated	4.6±0.29**	8.3±0.18**	3.7±0.06**	4.40±0.05**	0.491±0.03*

Paired Student's t-test. N=16.
**P<0.02; **P<0.001*

Table 3. The release of catecholamines and serotonin from discrete and isolated brain regions of 3-month-old male and female rats, untreated, sham operated or castrated at 3-weeks old

Table 4

	Amount of biogenic amine (nmoles/g tissue) emitted from the tissue within 20 min				
	Dopamine			**Norepinephrine**	**Serotonin**
MALES	**Striatum**	**Substantia nigra**	**Tuberculum olfactorium**	**Locus coeruleus**	**Raphe**
Vehicle (A)	6.6±0.23	11.8±0.23	6.8±0.21	9.6±0.19	1.178±0.14
Testosterone (B)	4.7±0.19	10.8±0.34	4.8±0.13	3.4±0.21	0.581±0.11
Estrone ©	5.8±0.21	11.6±0.26	5.8±0.20	4.2±0.35	0.918±0.04
	A:B ****	A:B *	A:B ****	A:B ****	A:B **
	A:C *	A:C ¯	A:C ***	A:C ****	A:C ¯
	B:C **	B:C ¯	B:C ***	B:C ¯	B:C *
FEMALES					
Vehicle (A)	7.7±0.27	11.8±0.26	7.9±0.17	9.0±0.26	1.120±0.07
Testosterone (B)	6.8±0.45	11.4±0.21	7.1±0.35	4.7±0.37	0.815±0.09
Estrone ©	5.5±0.16	11.2±0.39	6.3±0.39	3.7±0.32	0.377±0.11
	A:B ¯	A:B ¯	A:B ¯	A:B ****	A:B *
	A:C ****	A:C ¯	A:C ***	A:C ****	A:C ***
	B:C ***	B:C ¯	B:C ¯	B:C ¯	B:C *

Paired Student's t-test. N=16.
*¯P>0.05; *P<0.05; **P<0.02; *** P<0.01; **** P<0.001*

Table 4. The release of catecholamines and serotonin from discrete and isolated brain regions of 4-week-old male and female rats, 24 hours after a single subcutaneous injection with oil (0.1 ml/rat), testosterone propionate (0.1 mg/rat) and estrone (0.01 mg/rat), respectively

Table 5

	Amount of biogenic amine (nmoles/g tissue) emitted from the tissue within 20 min				
	Dopamine			**Norepinephrine**	**Serotonin**
MALES	**Striatum**	**Substantia nigra**	**Tuberculum olfactorium**	**Locus coeruleus**	**Raphe**
Vehicle	5.9±0.27	10.4±0.22	6.2±0.31	9.9±0.70	1.071±0.11
Progesterone	5.7±0.20	10.6±0.33	5.9±0.08	10.0±0.05	1.026±0.07
	P>0.05	*P*>0.05	*P*>0.05	*P*>0.05	*P*>0.05
FEMALES					
Vehicle	5.8±0.13	10.5±0.29	6.4±0.21	10.8±0.10	1.080±0.02
Progesterone	5.8±0.15	10.1±0.30	6.2±0.22	10.4±0.80	1.470±0.03
	P>0.05	*P*>0.05	*P*>0.05	*P*>0.05	*P*>0.05

Paired Student's t-test. N=16.

Table 5. The release of catecholamines and serotonin from discrete and isolated brain regions of 4-week-old male and female rats, 24 hours after a single subcutaneous injection with oil (0.1 ml/rat) and progesterone (0.5 mg/rat), respectively

Table 6

	Amount of biogenic amine (nmoles/g tissue) emitted from the tissue within 20 min				
	Dopamine			**Norepinephrine**	**Serotonin**
MALES	**Striatum**	**Substantia nigra**	**Tuberculum olfactorium**	**Locus coeruleus**	**Raphe**
Vehicle (A)	6.2±0.24	11.9±0.37	7.1±0.18	9.5±0.20	0.914±0.04
Testosterone (B)	5.0±0.17	11.8±0.10	5.1±0.13	5.8±0.17	0.281±0.01
Estrone ©	4.9±0.31	11.7±0.24	4.7±0.17	4.3±0.10	0.459±0.02
	A:B ***	A:B ¯	A:B ****	A:B **	A:B ***
	A:C *	A:C ¯	A:C ****	A:C ***	A:C ***
	B:C ¯	B:C ¯	B:C ¯	B:C ¯	B:C **
FEMALES					
Vehicle (A)	6.6±0.22	12.0±0.20	6.5±0.25	9.3±0.30	0.944±0.04
Testosterone (B)	3.4±0.13	10.9±0.23	4.6±0.26	5.2±0.05	0.236±0.02
Estrone ©	5.4±0.11	10.3±0.11	5.9±0.18	5.0±0.05	0.520±0.01
	A:B ****	A:B ***	A:B ***	A:B ***	A:B ***
	A:C ***	A:C ****	A:C ¯	A:C ***	A:C ***
	B:C ****	B:C ¯	B:C ***	B:C ¯	B:C ***

Treatment started on 3-week-old rats. Brain samples were isolated 24 hours after the last injection

Paired Student's t-test. N=16. ¯P>0.05; *P<0.05; **P<0.02; *** P<0.01; **** P<0.001

Table 6. The release of catecholamines and serotonin from discrete and isolated brain regions of 4-week-old male and female rats injected once daily for 7 days subcutaneously with oil (0.1 ml/rat), testosterone propionate (0.1 mg/rat) and estrone (0.01 mg/rat), respectively

Table 7

	Amount of biogenic amine (nmoles/g tissue) emitted from the tissue within 20 min				
	Dopamine			**Norepinephrine**	**Serotonin**
MALES	**Striatum**	**Substantia nigra**	**Tuberculum olfactorium**	**Locus coeruleus**	**Raphe**
Vehicle (A)	5.8±0.24	14.3±0.30	7.6±0.13	6.5±0.40	1.090±0.01
Testosterone (B)	6.4±0.28	13.0±0.19	5.8±0.24	5.6±0.10	0.415±0.01
Estrone ©	4.6±0.21	9.8±0.27	5.6±0.21	2.0±0.10	0.213±0.02
	A:B ¯	A:B ***	A:B ****	A:B ¯	A:B ***
	A:C **	A:C ***	A:C ****	A:C ***	A:C ***
	B:C ***	B:C ****	B:C ¯	B:C ***	B:C **
FEMALES					
Vehicle (A)	5.1±0.06	11.7±0.13	6.2±0.15	6.7±0.25	1.007±0.01
Testosterone (B)	4.4±0.18	10.8±0.36	4.5±0.15	3.8±0.15	0.218±0.02
Estrone ©	5.7±0.23	10.2±0.34	5.6±0.20	6.5±0.30	0.607±0.01
	A:B ***	A:B ¯	A:B ****	A:B ***	A:B ****
	A:C ¯	A:C ***	A:C *	A:C ¯	A:C ****
	B:C ***	B:C ¯	B:C ***	B:C **	B:C ***

Treatment started on 3-week-old rats. Brain samples were isolated 24 hours after the last injection. Paired Student's t-test. N=16.
¯$P>0.05$; *$P<0.05$; **$P<0.02$; *** $P<0.01$; **** $P<0.001$

Table 7. The release of catecholamines and serotonin from discrete and isolated brain regions of 4-week-old male and female rats injected once daily for 14 days subcutaneously with oil (0.1 ml/rat), testosterone propionate (0.1 mg/rat) and estrone (0.01 mg/rat), respectively.

1.8. First evidence supporting the unique beneficial CAE effect of DEP in humans: DEP-treatment of *de novo* parkinsonians significantly delayed the need for levodopa therapy (DATATOP Study 1987-1992). *It was the DATATOP study which clearly proved that DEP has a beneficial influence on the natural history of PD. DEP treatment significantly delayed the time in de novo parkinsonians until enough disability developed to warrant the initiation of levodopa therapy.*

Although the Parkinson Study Group organized the DATATOP study believing that DEP would benefit this multicenter clinical trial because it inhibits selectively MAO-B, the outcome presented clear-cut evidence that the CAE effect of DEP was fully responsible in changing the course of the disease in *de novo* parkinsonians.

It is remarkable that despite the unequivocal experimental evidence, the fact that DEP is primarily a PEA-derived CAE substance is ignored. Today, clinicians still classify DEP, at present the only synthetic CAE substance in clinical use, as merely a selective inhibitor of B-type MAO.

The CAE effect is unrelated to the selective inhibition of MAO-B. Both selective inhibitors of MAO-B registered after selegiline for clinical use (lazabemide: see Miklya and Knoll 2003; and rasagiline: see Miklya 2011, 2014b) are devoid of the enhancer effect. Moreover, convincing animal experiments speak in favor for the conclusion that DEP slows the rate of the functional deterioration of the nigrostriatal dopaminergic neurons, and the experimental findings are congruent with clinical evidence that DEP slows the progress of PD.

The indication for using DEP in patients with early, untreated PD was established in the DATATOP study in the USA (Tetrud and Langston 1989; Parkinson Study Group 1989, 1993). Important multicenter studies such as, the French Selegiline Multicenter Trial (FSMP) (Allain et al. 1991), the Finnish Study (Myttyla et al. 1992), the Swedish Parkinson Study Group (Palhagen et. al. 1998), and the Norwegian-Danish Study Group (Larsen et al. 1999) confirmed the usefulness of the drug in *de novo* PD.

Age-related deterioration of the striatal machinery is a continuum and any precisely determined short segment of it is sufficient to measure the rate of decline in the presence or absence of DEP. As a matter of fact, in the DATATOP multicenter study by the Parkinson Study Group, a segment of this continuum, the time elapsing from diagnosis of PD until levodopa was needed, was properly measured in untreated patients with PD and the effect of DEP versus placebo was compared (Parkinson Study Group 1989). Due to the continuous further

deterioration of the nigrostriatal dopaminergic neurons, usually within one year after the diagnosis of the disease, the patients need dopamine-substitution (levodopa therapy).

Among the participants of the DATATOP multicenter study Tetrud and Langston were the first who realized that DEP-treatment affects beneficially the natural history of PD. In 1989, they published that DEP delays the need for levodopa therapy. In their study, the average time that elapsed before levodopa was needed was *312.1 days* for patients in the placebo group and *548.9 days* for patients in the DEP group (Tetrud and Langston 1989).

DEP**, in the dose which blocks MAO-B activity in the brain, **exerts its non-specific enhancer effect (Knoll 2012; Chapter 9, Fig.18; and see in this essay Fig 12), and keeps via this mechanism the surviving dopaminergic neurons on a higher activity level. Since rasagiline, selective inhibitor of B-type MAO, is devoid of the CAE effect and did not delay the levodopa need like DEP, it is obvious that the non-specific CAE effect is fully responsible for the unexpected finding, first published in *Science* by Tetrud and Langston in 1989 and in the same year confirmed by the Parkinson Study Group in *New England Journal of Medicine*.

The design of the DATATOP study was unintentionally similar that we had used in our rat experiments with DEP since 1980. We tested the sexual activity of male rats as a quantitatively measurable, rapidly aging dopaminergic function, and compared the effect of DEP versus saline treatment on the age-related decline of sexual potency in male rats. We demonstrated that DEP-treatment significantly slowed the age-related decay of sexual performance (Knoll 1982) and later went on to show that this effect of DEP was unrelated to the inhibition of MAO-B (Knoll 1987). To play the best card in support to this conclusion, we performed a structure-activity-relationship (SAR) study which aimed to select a derivative of DEP that was free of MAO inhibitory property (Knoll et al. 1992a).

In DEP, the propargyl group binds covalently to the flavin of MAO-B, and this leads to the irreversible inhibition of the enzyme activity. (-)-1-Phenyl-2-propylaminopentane [(-)-PPAP], the new DEP selected analogue, differed from its mother compound by containing a propyl group instead of a propargyl group. As expected, this compound enhanced dopaminergic activity in the brain like DEP, but did not change the activity of MAO-B. One can follow the progress in clarifying the mechanism in DEP responsible for enhanced dopaminergic activity by referring to a series of papers and reviews (Knoll 1978, 1983, 1987, 1992a,b, 1995, 1998, 2001, 2003).

Today, it is clear that if we select a quantitatively measurable dopaminergic function and determine its age-related decline by fixing an exact end, there is a shift of this end stage in time in DEP-treated rats which shows the DAE effect of the drug. For example, due primarily to the physiological aging of the striatal dopaminergic system, male rats completing their second year of age lose completely their ability to ejaculate.

We found that saline-treated Wistar (Charles River) male rats from our breed reached this stage at the age of 112±9 weeks, whereas their DEP-treated peers lost the ability to ejaculate only at the age of 150±12 weeks (P <0.001) (Knoll 1993). The design of the DATATOP study was essentially the same. The authors knew that after having diagnosed PD the next step would be the need for levodopa, and they measured the DEP-induced delay in reaching this stage.

The authors of the DATATOP study expected DEP to be efficient in their trial because of its MAO-B inhibitory effect. Their hypothesis was that the activity of MAO and the formation of free radicals predispose patients to nigral degeneration and contribute to the emergence and progression of PD. In accord with their working hypothesis they expected that DEP, the MAO inhibitor, α-tocopherol, the antioxidant, and the combination of the two compounds will slow the clinical progression of the disease because MAO activity and the formation of oxygen radicals contribute to the pathogenesis of nigral degeneration. They selected patients with early, untreated PD and measured the delay in the onset of disability necessitating levodopa therapy.

In the first phase of the trial, 401 subjects were assigned to α-tocopherol or placebo and 399 subjects were assigned to DEP, alone or with α-tocopherol. Only 97 subjects who reached the 'end' of the trial (i.e., the onset of disability necessitating levodopa therapy) during an average 12 months of follow-up compared with 176 subjects who did not receive DEP. The risk of reaching the end of the trial was reduced by 57% for the subject who received DEP, and these patients also had a significant reduction in their risk of having to give up full-time employment (Parkinson Study Group 1989). Following the course of changes, the authors concluded in their next paper (Parkinson Study Group 1993) that DEP, but not α-tocopherol, delayed the onset of disability associated with early, otherwise untreated PD. But as time passed, the DATATOP study also revealed that DEP did not reduce the occurrence of subsequent levodopa-associated adverse effects in the patients (Parkinson Study Group 1996).

The real therapeutic value of DEP in the case of PD is that with a safe small daily dose (1mg), taken from sexual maturity, the dopaminergic neurons are maintained on a higher

activity level, thus the chance to avoid the manifestation of PD is significantly increased with the enhancer-protection. Despite convincing experimental evidence, so far, the exact clinical analysis test needed has regrettably failed to come about.

The unexpected outcome of the DATATOP study clearly indicated that DEP possesses an unknown pharmacological effect of basic importance and α-tocopherol is devoid of this effect. We succeeded in the 1990s to explain the ineffectiveness of α-tocopherol in the DATATOP study. As shown in detail earlier, we demonstrated that PEA and tyramine are not only well-known releasers of catecholamines from their intraneuronal pools, but they are primarily CAE substances. They enhance in low doses the impulse propagation mediated release of catecholamines. As shown in Fig.8 the catecholamine-releasing property of these amines concealed the CAE effect, the physiologically important property of these amines remained undetected (Knoll et al. 1996a).

As expected a comparison of the enhancer effect of α-tocopherol with that of DEP showed that α-tocopherol did not change the impulse-evoked release of catecholamines in the brain; thus the compound is devoid of the CAE effect (Miklya et al. 2003, 2014b). This is clear proof that the CAE effect was responsible for the effectiveness of DEP in the DATATOP study (as a recent review see Miklya 2011).

The clinical trial with rasagiline, performed by the Parkinson Study Group, revealed that unlike the early DEP trials, rasagiline failed to demonstrate a decreased need for levodopa (Parkinson Study Group 2002). Even the results of a couple of recent studies (Olanow and Rascol 2010; Ahlskog and Uitti 2010; Mehta et al. 2010) led to the conclusion that "based on current evidence, rasagiline cannot be said to definitely have a disease-modifying effect" (Robottom 2011). It is congruent with this finding that rasagiline is devoid of the CAE effect (Miklya 2011, 2014b).

We have to consider the physiological role of the nigrostriatal dopaminergic neurons in the continuous activation of the cerebral cortex. It is common knowledge that this is realized via a highly complicated route of connections. The neostriatum is the main input structure of the basal ganglia. It receives glutamatergic input from many areas of the cerebral cortex. Cholinergic and peptidergic striatal interneurons are in connection with the nigrostriatal dopaminergic neurons. Dopamine released in the striatum controls the two GABAergic pathways along which the outflow of the striatum proceeds. One is a direct route to the substantia nigra pars compacta and medial globus pallidus. The other is an indirect route. A

GABAergic link binds the striatum to the lateral globus pallidus; from here, another GABAergic pathway goes to the subthalamic nucleus, which provides glutamatergic excitatory innervation to the substantia nigra pars compacta and medial globus pallidus. This then continuously inhibits - via a GABAergic projection - the activity of the ventroanterior and ventrolateral nuclei of the thalamus, which provide feedback glutamatergic excitatory impulses to the cerebral cortex.

Thus, the stimulation of the direct pathway at the level of the striatum increases the excitatory outflow from the thalamus to the cortex, whereas stimulation of the indirect pathway has the opposite effect. The striatal GABAergic neurons of the direct pathway express primarily the excitatory D_1 dopamine receptors; the striatal neurons of the indirect pathway express primarily the inhibitory D_2 receptors. As a result dopamine release in the striatum increases the inhibitory activity of the direct pathway and diminishes the excitatory activity of the indirect pathway. As a net effect, the inhibitory influence of the substantia nigra pars reticulata and medial globus pallidus on the ventroanterior and ventrolateral nuclei of the thalamus is reduced, thus increasing the excitatory effect of these nuclei on the cerebral cortex. All in all, ***a more active nigrostriatal dopaminergic system means a more active cerebral cortex and, vice versa.*** Because of the well-known, highly complicated, key-important nature of this system and because, as discussed in detail earlier (Knoll 2012), with the discovery of the enhancer sensitivity of the catecholaminergic and serotonergic neurons we just see the peak of the iceberg. We analyze now the nigrostriatal dopaminergic brain machinery as a model to detect hitherto unknown enhancer sensitive regulations.

Since the physiological age-related decline of the nigrostriatal dopaminergic activity leads necessarily to an equivalent reduction in the activity of the cerebral cortex, this change plays a significant role in the decline of performances over time. Aging of the dopaminergic system in the brain plays an undisputable leading role in the highly significant, substantial decline in male sexual activity and also in the more modest but still significant age-related decline in learning performance.

According to a human male study, median coital activity was the highest, 2.1 events/week between the ages of 30 and 34. This rate decreased progressively with increasing age, sinking to 0.2/week ($P<0.001$) in the 65 to 69-year-old age group (Martin 1977). We found essentially the same trend of changes in male rats in series of different experiments (Knoll 1988, 1989; Knoll et al. 1983, 1994).

There is a quantitative difference only between the physiological age-related decline of the dopaminergic input and that observed in PD. In the healthy population, the calculated loss of striatal dopamine is about 40% at the age of 75, which is about the average lifetime. The loss of dopamine in PD is 70% or thereabout at diagnosis and over 90% at death. The drastic reduction of the dopaminergic output in PD evidently leads to an accordingly drastic reduction of cortical activity and *this makes it clear why an enhancer substance, like DEP, improves cognition, attention, memory and reaction times. It also brings about subjective feelings of increased vitality, euphoria and increased energy in people with PD* (Knoll 2012).

In diagnosing PD, the neurologist selects subjects with the most rapidly aging striatal dopaminergic system (about 0.1% of the population). As symptoms of PD become visible only after the unnoticed loss of a major part (about 70%) of striatal dopamine and further deterioration is irresistible, the disease is, in this sense, incurable.

It is obvious that with the progression of the disease the chances to enhance the activity of the dopaminergic neurons via the administration of a synthetic enhancer substance are going from bad to worse. In a recent study the clinical outcome of PD patients treated with DEP plus levodopa was evaluated in the early stage of the disease in comparison with that of late-stage use of only DEP. The clinical outcome, as evaluated by the selected Unified PD Rating Scale (UPDRS) motor scores was better for levodopa-treated patients who received DEP within 5 years from the onset compared with those who received DEP approximately 10 years from the onset (Mizuno et al. 2010). On the other hand, a recent study confirmed even that patients who were treated with DEP for 3 or more years in early PD showed a slower progression of the disease, as evaluated by the Hoen and Yahr Stage transition times (Zhao et al. 2011).

Due to the inhibition of MAO-B, DEP treatment allows for a 20-50% decrease in levodopa dose needed in PD. In patients who need levodopa, however, there is always a risk that the administration of DEP will enhance the side effects of levodopa which can only be avoided by properly decreasing the levodopa dose according to the individual sensitivity of the patient.

The Parkinson's Disease Research Group of the United Kingdom (PDRG-UK) used an improper combination of levodopa and selegiline which led to confusion and misinterpretation.

Quite unexpectedly, this group published an alarming paper claiming that parkinsonian patients treated with levodopa combined with DEP show an increased mortality in comparison with the patients treated with levodopa alone (Lees 1995). This finding was in striking

contradiction to all other studies published in a variety of countries. The "idiosyncratic prescribing" (Dobbs et al. 1996) of DEP in combination with levodopa in the PDRG-UK study led to false conclusions by the authors. Comments uniformly pointed to the substantial overdosing of levodopa as the cause of the observed deaths with DEP as an adjuvant in this trial (Dobbs et al. 1996; Knoll 1996; Olanow et al. 1996).

Since in the mid-1980s , when we realized that DEP prolongs the life of rats, Birkmayer in Vienna was the only clinician who treated for nearly a decade hundreds of parkinsonians with Madopar+DEP and Madopar alone, we agreed to make a retrospective analysis to see how the addition of DEP changed life expectancy in humans. In an open, uncontrolled study the long-term (9 years) effect of treatment with Madopar alone (N=377) or in combination with DEP (N=564) was compared. *The survival analysis revealed a significant increase in life expectancy in Madopar combined DEP group (Birkmayer et al. 1985).*

Shoulson summarized the results of the DATATOP study. Beginning in 1987, the study was conducted at 28 academic medical centers in the United States and Canada. After an average of 8.2 years of observation, the overall death rate of the subjects was 17.1% (137 of 800) or 2.1%/year. The final conclusion was that selegiline (10 mg/day) significantly delayed the time until enough disability developed to warrant the initiation of levodopa therapy. The effect was largely sustained during the overall 8.2 years of observation. ***Tocopherol produced no benefits!*** *The 2.1% per year mortality rate of the DATATOP cohort was remarkably low, about the same as an age-matched population without PD* (Shoulson 1998).

Considering the peculiar pharmacological profile of DEP, the unusual safety of this drug and the incurable nature of PD and AD, it is unfortunate that we are still awaiting a multicenter, controlled clinical trial, designed to measure the prevalence of these neurodegenerative diseases in a cohort treated from at least 60 years old with 1 mg selegiline daily.

An inverse correlation between brain DA loss in PD and tissue NE levels regarding the potential neuroprotective effect of preventive DEP medication against the manifestation of PD was described (Tong et al. 2006). Since selegiline, as a CAE substance, keeps the NE levels in the brain higher, this effect of preventive DEP medication might be an additional factor that works against the manifestation of PD.

1.9. The peculiar bi-modal, bell-shaped concentration effect curve characteristic to the "specific" and "non-specific" CAE effect of DEP. The dose of DEP used to fully inhibit B-

type monoamine oxidase (MAO-B) activity in the brain is also the optimum dose for the non-specific CAE effect. *A bi-modal, bell-shaped concentration effect curve is characteristic to the CAE effect of the enhancer substances.* We took first notice of this peculiar behavior in the course of our first experiments when we realized the CAE effect of DEP (Knoll and Miklya 1994). Nevertheless, only the exact analysis of the enhancer effect of BPAP, the selective and up to the present most potent enhancer substance, rendered the unquestionable distinction of the specific and non-specific enhancer effect from each other possible. The bipolar, bell-shaped nature of the enhancer effect was first verified on cultured rat hippocampal neurons (Knoll et al. 1999) and more exactly analyzed on isolated locus coeruleus of rats (Knoll et al. 2002).

To demonstrate this peculiar behavior of DEP in *in vivo* experiments we analyzed in a modified version of the shuttle box (originally described by Bovet et al. 1966) the acquisition of a two-way conditioned avoidance reflex (CAR) during 5 consecutive days. Rats were trained with 100 trials per day in a box divided inside into two parts by a barrier with a small gate in the middle. One trial consisted of a 10s inter-trial interval followed by 20s conditioned stimulus (CS, light flash), the last 5s of which was overlapped with a foot shock (1mA), the unconditioned stimulus (US). The rat learns to avoid punishment and escapes in response to flash light within 15s (CAR). If the rat failed to respond within 5s to the US, this was noted as an escape failure (EF). At each learning session, the number of CARs, EFs and intersignal reactions (IRs) are automatically counted and evaluated by multi-way ANOVA. Tetrabenazine (T) or T+DEP were injected subcutaneously 1 hour prior to training.

Tetrabenazine-treatment (1 mg/kg sc.), which blocks the vesicular monoamine transporter 2 (VMAT2), depletes at least 90% of NE and DA from their stores in the nerve terminals of the catecholaminergic neurons in the brain stem. Due to the weak performance of the catecholaminergic brain engine, the activation of the cortical neurons remains below the level required for the acquisition of a CAR. According to our experience (we studied through years the drug families worth trying), tetrabenazine-induced inhibition of learning performance can only be antagonized by administration of a synthetic CAE substance or by the complete inhibition of A-type MAO , whereas selective inhibition of B-type MAO or inhibition of the reuptake of catecholamines and/or serotonin is ineffective (Knoll et al. 1992a).

Figure 12

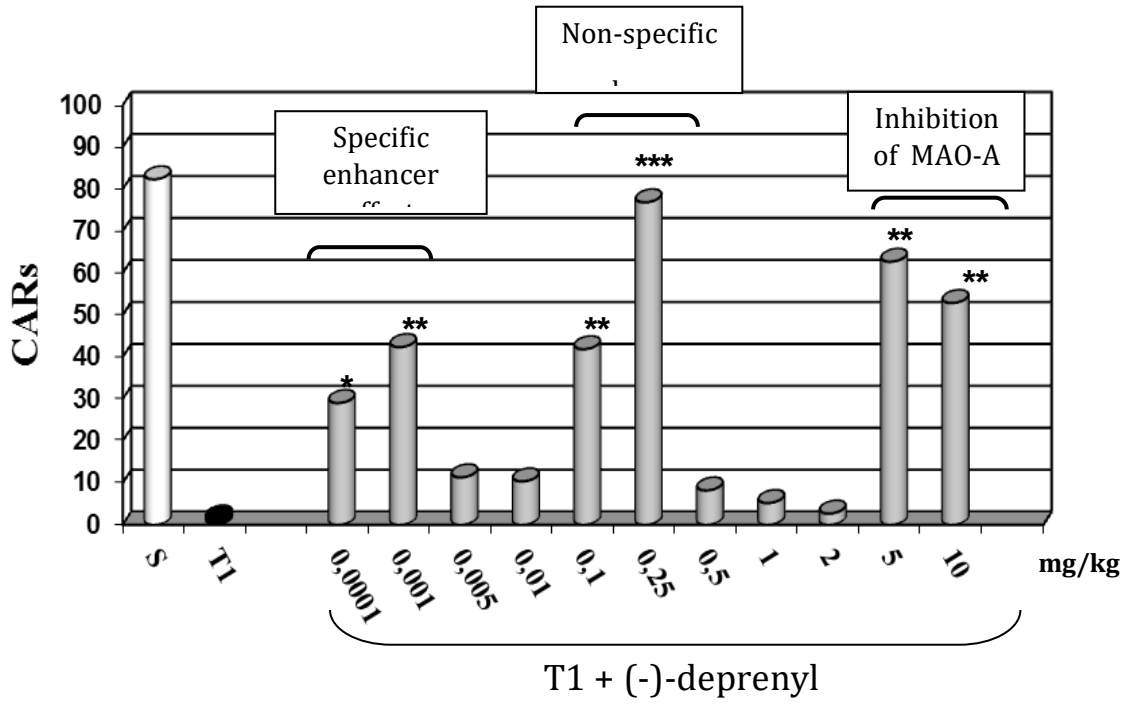


Fig.12. Antagonism of tetrabanazine-induced inhibition of learning performance in the shuttle box by DEP in the bi-polar, bell-shaped manner characteristic to the CAE substances. Measured: (S) the ability of saline-treated (control) rats to fix conditioned avoidance responses (CARs); (T1) the inhibition of the learning ability of rats treated subcutaneously with 1 mg/kg tetrabenazine, one hour prior to training; [T1 + DEP] the ability of DEP to antagonize in a dose related manner the inhibitory effect of tetrabenazine. Significance in the performance between the groups was evaluated by multi-factor analysis of variance (ANOVA). *P<0.05; **P<0.01, ***P<0.001

Since DEP, the first selective inhibitor of B-type MAO is classified from the 1970s in all papers and textbooks as the reference compound to block this enzyme, its CAE effect, the real significance of which was realized only in the late 1990s, remained unfortunately neglected. Fig.13 clearly demonstrates that the dose of DEP used in animal experiments and in humans as the optimum choice one to fully block MAO-B is also the optimum dose to elicit the *non-specific CAE effect*. ***It remains for the future to find out the role of the non-specific CAE effect in the therapeutic benefits observed for decades in DEP-treated patients***.

1.10. First longevity study showing that treatment with low dose of DEP which blocks MAO-B activity in the brain and exerts its non-specific enhancer effect prolongs life significantly. *In the belief that selective inhibition of B-type MAO is fully responsible for the beneficial pharmacological effects of DEP, we performed prior to the discovery of the CAE effect, two longevity studies with the 0.25 mg/kg dose which blocks completely MAO-B activity in the rat brain.*

We experienced in our behavioral studies performed since 1955 the validity of the common concept that there is a great individual variation in sexual activity and learning performance in any random population of mammals of the same strain. For example, in our second longevity study (Knoll et al. 1994) we selected from a population of sexually inexperienced 1600 Wistar-Logan rats the ones with the lowest sexual potency and found 94 rats which did not display in four consecutive weekly mating tests any sign of sexual activity. We observed their sexual activity in the presence of a female with high receptivity during 30 minutes and counted the copulatory patterns of the male (mounting, intromission and ejaculation). The "non-copulators" remained inactive until they died. On the other hand, we found 99 males which displayed at least one ejaculation in each of the four tests.

The discovery of the bell-shaped concentration/effect curve of the enhancer substance, in the low nano-molar concentration range, offers the first reasonable explanation for the great individual variation in behavioral performances. Since an *optimum* concentration of the enhancer substance was needed for the *optimum* performance, *I postulate that the substantial individual differences in behavioral performances are due to the peculiar dose-dependency of the endogenous enhancer substances.* This approach granted us a new perspective on the results of our two longitudinal studies performed on rats (first longevity study: Knoll 1988; Knoll et al. 1989; second longevity study: Knoll et al. 1994).

In the years when we performed our two longevity studies and worked with the robust Wistar-Logan rats, we observed that the males which completed the second year of their life did never display in the weekly mating test a single ejaculation. We experienced later that the Sprague-Dawley CFY or Wistar (Charles-River) rats too lost this ability at this age. Because of the crucial importance of the message it was already mentioned earlier (PART 1.1) that the aging-related natural decay of the dopaminergic brain machinery is responsible for this change. *Saline-treated CFY male rats reached the stage of inability to ejaculate at an average of **112±9 weeks**, their DEP-treated peers, due to the DAE effect of DEP, reached that stage at an average of **150±12 weeks** (Knoll 1993).*

In our first longevity study we started to work with 132 sexually inexperienced

2-year old males, thus we tested first their sexual activity in four consecutive weekly mating tests during the 24th month of their life. According to their screening the rats were divided in three groups: 46 "non-copulators", 42 "mounting" rats and 44 "sluggish" rats (displaying mountings and intromissions). Thereafter we treated 66 rats with saline and 66 rats with 0.25 mg/kg (-)-deprenyl, three times a week, and observed their behavioral performances to the end of their life. We observed the rats until death.

The saline-treated group of the "non-copulators" died out first, the "mounting" rats lived longer, and the longest living rats were in the "sluggish" group (see Table VI in Knoll 1988). DEP treatment prolonged the life in each group significantly. The 66 salt-treated rats lived in average *147.05± 0.56 weeks*, the 66 (-)-deprenyl-treated rats lived in average *197.98± 2.31 weeks*.

The fact that the saline-treated "non-copulators" died out first and the finding that DEP, which keeps the catecholaminergic neurons on a higher activity level, prolongs their life, suggested that the catecholaminergic engine of the brain, the crucial important activator of the cortex, is responsible for the lifespan-prolonging effect. Thus, the brain engine works in the 2-year old "non-copulator" males on a lower activity level than in the 2-year old "sluggish" males. This working hypothesis, based on the results of the first longevity study, determined the planning of the second longevity study *We decided to start the experiment with younger rats, select from a huge population of Wistar-Logan rats the "non-copulators" and the sexually most active males, measure their sexual potency and learning ability until the end of their life, and treat the rats with saline and DEP, respectively.*

We started working with a random population of 28-week-old male rats and tested their sexual performance once a week. Rats that represented the two extremes in performance were selected for the study: the ones that did not display a single intromission during the four consecutive weekly-mating tests used for selection, and the ones which showed full scale sexual activity (mounting, intromission, ejaculation) in each of the four tests. Out of 1,600 sexually inexperienced 28-week-old Wistar-Logan male rats, that met a receptive female once a week during four consecutive weeks, ***94*** *did not display a single intromission during the selection period and* **99** *displayed at least one ejaculation in each of the four tests. The former were taken for the* ***lowest performing (LP)*** *rats and the latter for the* ***highest performing (HP)*** *ones*.

After selection we started to treat the 8-month-old rats subcutaneously with either 1 ml/kg 0.9% NaCl or with 0.25 mg/kg DEP, dissolved in 0.9% NaCl given in the same volume,

three times a week, until death. Out of the 94 LP animals, 46 were treated with saline. Out of the 99 HP animals, 49 were treated with saline. The mating and learning performances of these saline-treated LP and HP rats were tested during a period of 108 weeks. Sexual activity was tested once a week. The learning performance of the rats was tested in the shuttle box. The rats were trained once every three months for a period of five days, with 20 trials a day. In this longevity study we trained our rats in the shuttle box instead of the optimal training conditions (100 trial), only with 20 trials, to find more pronounced difference in the learning ability between high and low performing rats.

We found a highly significant difference in sexual and learning performances and in life span between LP and HP rats. The saline-treated LP rats (n=44) never displayed ejaculation during their lifetime, they were extremely dull in the shuttle box and lived 134.58±2.29 weeks. The saline-treated HP rats (n=49) displayed 14.04±0.56 ejaculations during the first 36-week testing period and due to aging they produced 2.47±0.23 ejaculations between the 73-108th week of testing. They lived 151.24±1.36 weeks, significantly (P<0.001) longer than their LP peers.

Maintenance on DEP enhanced the performance of both LP and HP rats and prolonged their lifespan significantly. The DEP-treated LP rats (n=48) became sexually active, their mating performance was substantially increased and lived 152.54±1.36 weeks, significantly longer than their saline-treated peers and as long as the saline-treated HP rats. The DEP-treated HP rats (n=50) were sexually much more active than their saline-treated peers. They displayed 30.04±0.85 ejaculations during the first 36-week testing period and 7.40±0.32 ejaculations between the 73-108th week of testing. Also their learning performance was substantially increased. They produced 113.98±3.23 CARs during the first 36-week-testing period and 81.68±2.14 CARs during the 73-108th week of testing. They lived for 185.30±1.96 weeks, significantly longer than their salt-treated peers; and out of the 50 rats *17 lived longer than the maximum lifespan ever observed during a long observation period on hundreds of untreated or saline treated rats in the strain of Wistar Logan males used in our studies*.

Considering the unique dose-related effect of an enhancer substance, we assume that out of the 1600 rats, 99 HP rats produced their endogenous enhancer substances at the peak of the bell-shaped concentration/effect curve, while the 94 LP rats produced them at the least active part of the curve. The overwhelming majority of the population (1407 rats) falls between these two extremes.

An analysis of the ability of rats to acquire the glass-cylinder-seeking drive is another example that convincingly illustrates the great individual differences in the behavioral performances of rat (see Sect.1.3 and 4.1 in Knoll 2005). We observed only in two rats out of 100 that the acquired glass-cylinder-seeking function operated lifelong with unchanged intensity. Presumably the specific endogenous enhancer substances in the cortical neurons responsible for the operation of the glass-cylinder-seeking drive were mobilized in these two rats optimally. Thus, *we may look upon these two rats as the most talented ones in the tested population regarding the measured function*.

Since those born with a healthy brain are equally provided with about 100 billion neurons and 10^{11} bit capacity, everybody has necessarily brilliant abilities which remain unexplored, unutilized (Knoll 2005).

1.11. First longevity study showing that treatment with low dose of DEP prolongs life. Based on our findings shown in Fig.12, we started the first longevity study with low doses of DEP in May 2010. We injected from two-month age, subcutaneously, 3-times a week (Monday, Wednesday and Friday) groups of male Wistar rats (N=40), with saline, 0.001 and 0.1 mg/kg DEP, respectively. Animals were observed until their natural death.

Figure 13

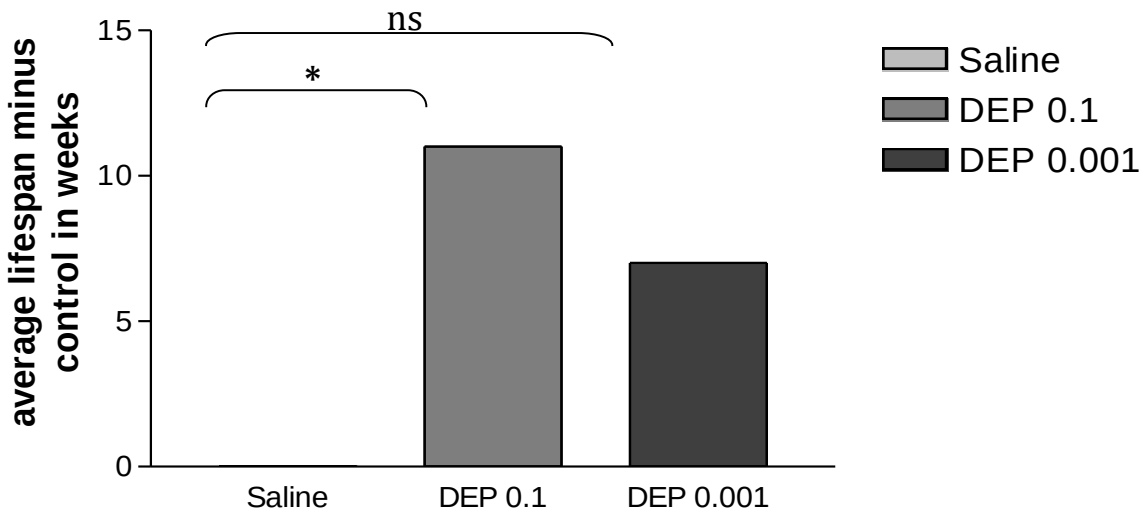


Fig.13 shows the average lifespan minus control in weeks in the groups treated with 0.1 and 0.001 mg/kg DEP, respectively. The rats treated with 0.1 mg/kg DEP lived significantly longer than their saline-treated peers. Even the rats treated with 0.001 mg/kg DEP lived longer than their saline treated peers, though the change was not statistically significant. ns=p>0.05; *p<0.05.

In harmony with Fig.13, there is a difference in the course of changes in survival between groups of saline and 0.1 mg/kg DEP (Fig.14) and between groups of saline and 0.001 mg/kg DEP (Fig.15), but according to the Kaplan-Meier statistical analysis the difference remains below the statistically significant level.

Figure 14

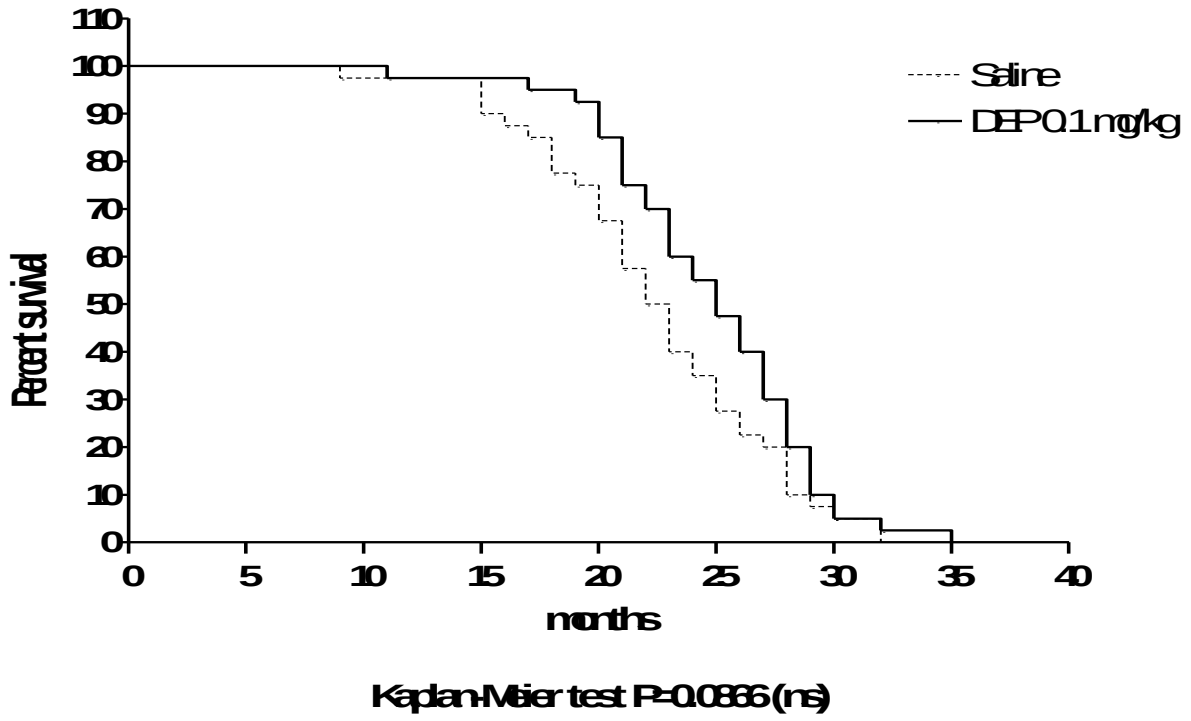


Fig.14. Difference in the course of changes in survival between groups of rats (N=40) treated with saline and 0.1 mg/kg DEP, respectively.

Figure 15

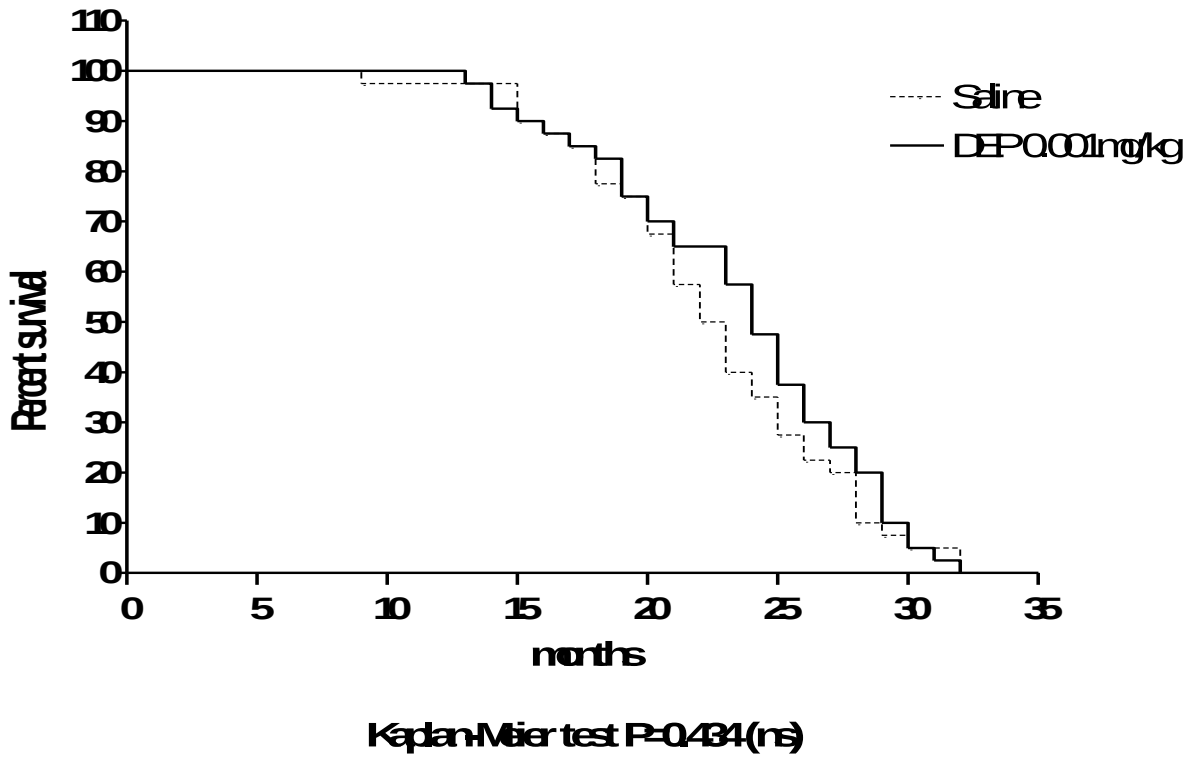


Fig.15. Difference in the course of changes in survival between groups of rats (N=40) treated with saline and 0.001 mg/kg DEP, respectively.

Table 8

TREATMENT	AVERAGE LIFESPAN IN WEEKS		
Saline	94.23 ± 3.48		[100%]
DEP 0.1 mg/kg	105.20 ± 3.07	(P<0.05)	[112%]
DEP 0.001 mg/kg	101.60 ± 3.38	(ns)	[108%]

Table 8. The average lifespan of rats in weeks, treated with 0.1 and 0.001 mg/kg DEP, respectively.

Table 9

TREATMENT	SHORTEST LIVING RAT (weeks)	LONGEST LIVING RAT (weeks)
Saline	36 [100%]	135 [100%]
DEP 0.1 mg/kg	46 [128%]	138 [102%]
DEP 0.001 mg/kg	61 [169%]	35 [100%]

Table 9. The shortest and longest living rats in the saline- and DEP-treated groups of rats, respectively.

Table 8. shows the average lifespan of rats treated with saline, 0.1 and 0.001 mg/kg DEP, respectively. The difference in the average lifespan between saline and 0.1 mg/kg DEP-treated rats was statistically significant ($P<0.05$).

Table 9 shows the shortest and longest living rat in the groups treated with saline, 0.1 and 0.001 mg/kg DEP, respectively. The shortest living rat lived in the 0.001 mg/kg DEP-treated group with 25 weeks longer and in the 0.1mg/kg treated rats with 10 weeks longer than the shortest living rat in the saline-treated group.

The data shown in Figures 12-15 and in Tables 8 and 9 are *indicative* of the role of the enhancer effect in the DEP-treatment induced prolongation of life. Since, as it will be shown in Part 2, even maintenance of rats on 0.0001 mg/kg BPAP, the much more potent enhancer substance than DEP, significantly prolonged the life of rats, it is obvious that the 3-times a week treatment with low doses of DEP was not sufficient to reach a statistically significant change, and a longevity study with a daily treatment with DEP is now in progress.

PART 2

Stages of the development of BPAP, the presently known most potent and highly selective synthetic enhancer substance

The finding that tryptamine is a CAE substance like PEA (Knoll 1994); and experimental evidence that the serotonergic neurons work with significantly enhanced activity in the brain from weaning until sexual maturity (Knoll and Miklya 1995), clearly indicated that, like PEA, tryptamine is also an endogenous enhancer substance.

2.1. The enhancer effect of tryptamine compared to PEA. Figures 16, 17 and 18 show the significant enhancement of the nerve-stimulation induced release of [^{3}H]-NA, [^{3}H]-DA, [^{3}H]-SER, respectively, from the rat's isolated brain stem in the presence of PEA and tryptamine.

Since a lower concentration of tryptamine (1.3 µmol/l) proved to be much more potent in enhancing the stimulation-evoked release of serotonin than a much higher concentration of PEA (16 µmol/l) (Fig.19), this suggests that, on a molecular level, the enhancer regulation in the catecholaminergic and serotonergic neurons are not identical.

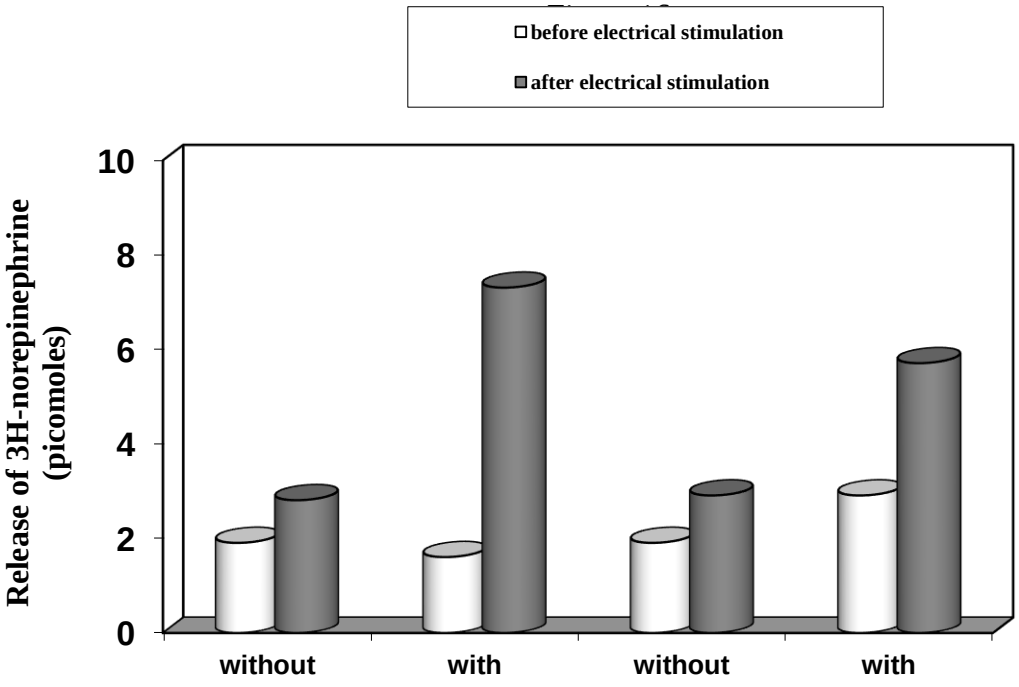
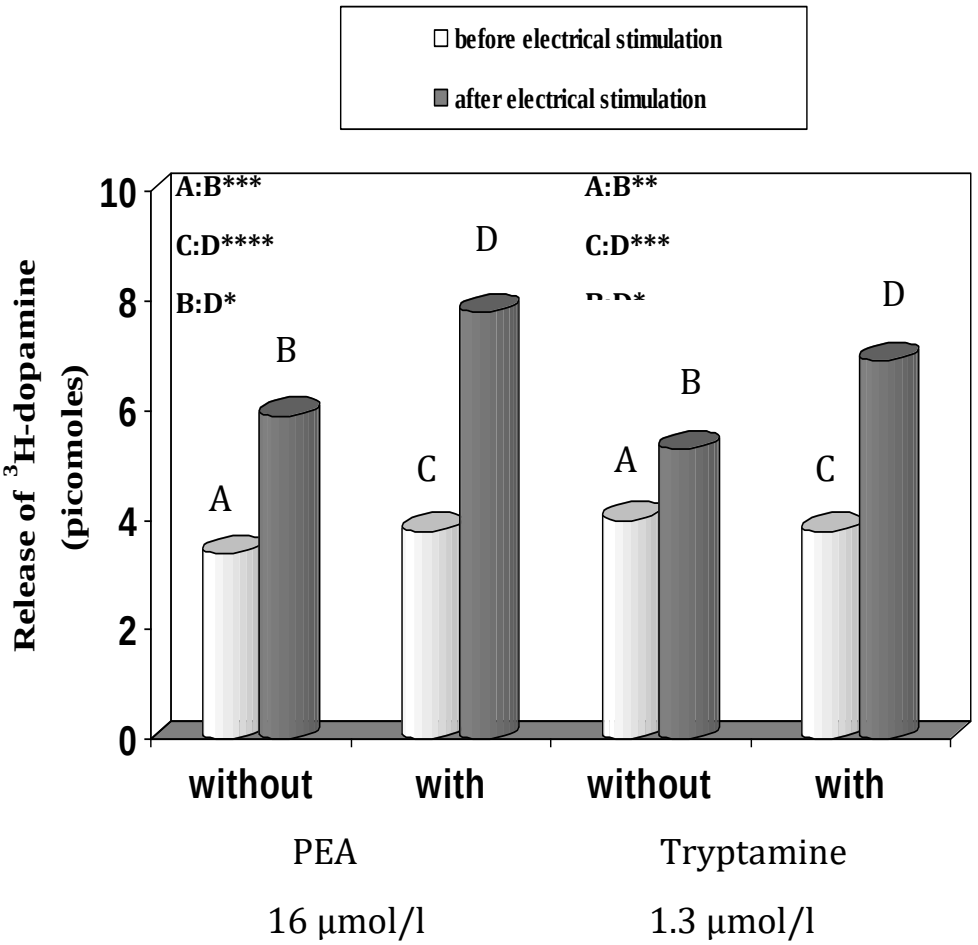


Fig.16. Significant enhancement of the nerve-stimulation-induced release of [^{3}H]-norepinephrine from the rat's isolated brain stem in the presence of □-phenylethylamine (PEA) and tryptamine, respectively (N=8). Each graph bar represents the amount of the labeled transmitter in picomoles released in a 3-min collection period. See Knoll et al. (1996a) for methodology. Paired Student's *t*-test was used for statistical analysis.

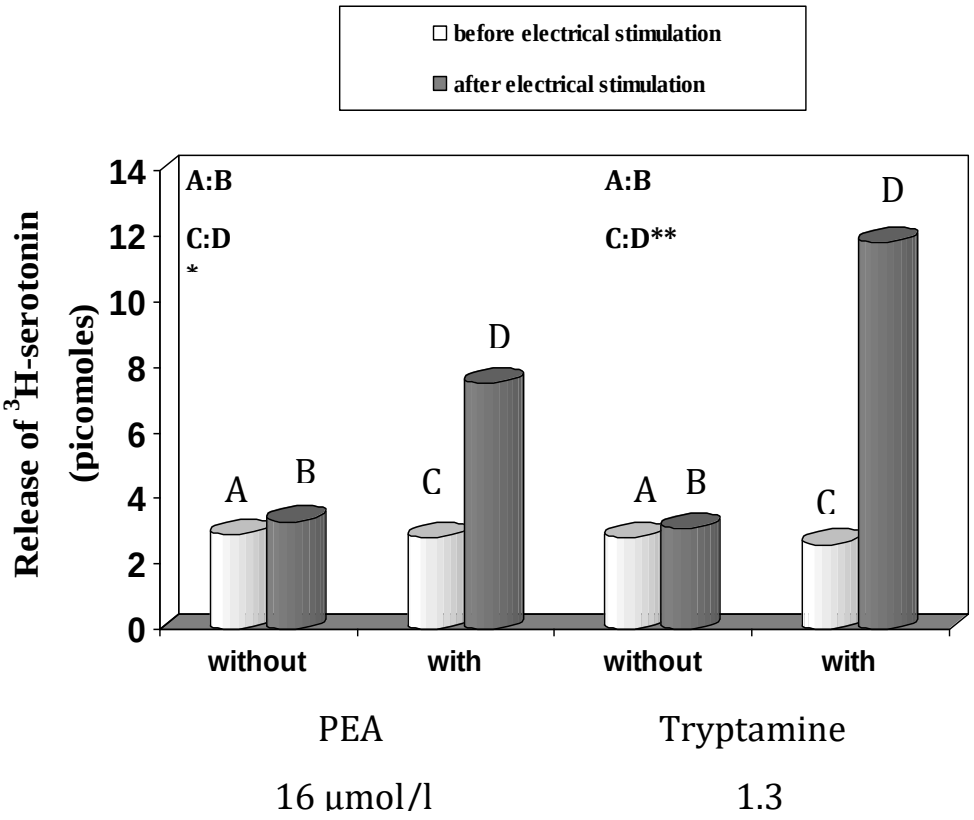
Figure 17



**P<0.05, **P<0.02, ***P<0.01, ****P<0.001.*

Fig.17. Significant enhancement of the nerve-stimulation-induced release of [^{3}H]-dopamine from the rat' s isolated brain stem in the presence of □-phenylethylamine (PEA) and tryptamine, respectively (N=8). Each graph bar represents the amount of the labeled transmitter in picomoles released in a 3-min collection period. See Knoll et al. (1996a) for methodology. Paired Student's *t*-test was used for statistical analysis.

Figure 18



*P<0.01, **P<0.001

Fig.18. Significant enhancement of the nerve-stimulation-induced release of [^{3}H]-serotonin from the rat's isolated brain stem in the presence of □-phenylethylamine (PEA) and tryptamine, respectively (N=8). Each graph bar represents the amount of the labeled transmitter in picomoles released in a 3-min collection period. See Knoll et al. (1996a) for methodology. Paired Student's *t*-test was used for statistical analysis.

2.2. Development of tryptamine-derived synthetic enhancer substances. The discovery that tryptamine is, like PEA, a natural enhancer substance (Knoll 1994), initiated the structure-activity-relationship study aiming to develop a new family of synthetic enhancer compounds; unrelated to PEA and the amphetamines. Of the newly synthesized compounds BPAP was selected for using it in the future as the reference substance for the analysis of the enhancer regulation in the mammalian brain (Knoll et al. 1999). Fig.19 shows the chemical structure and pharmacological spectrum of tryptamine; (-)-IPAP, the simplest tryptamine- derived, synthetic enhancer substance; and (-)-BPAP, the tryptamine-derived most representative enhancer substance. Fig.20 shows the IUPAC name and structure of BPAP, the presently known most

potent synthetic enhancer substance.

Figure 19

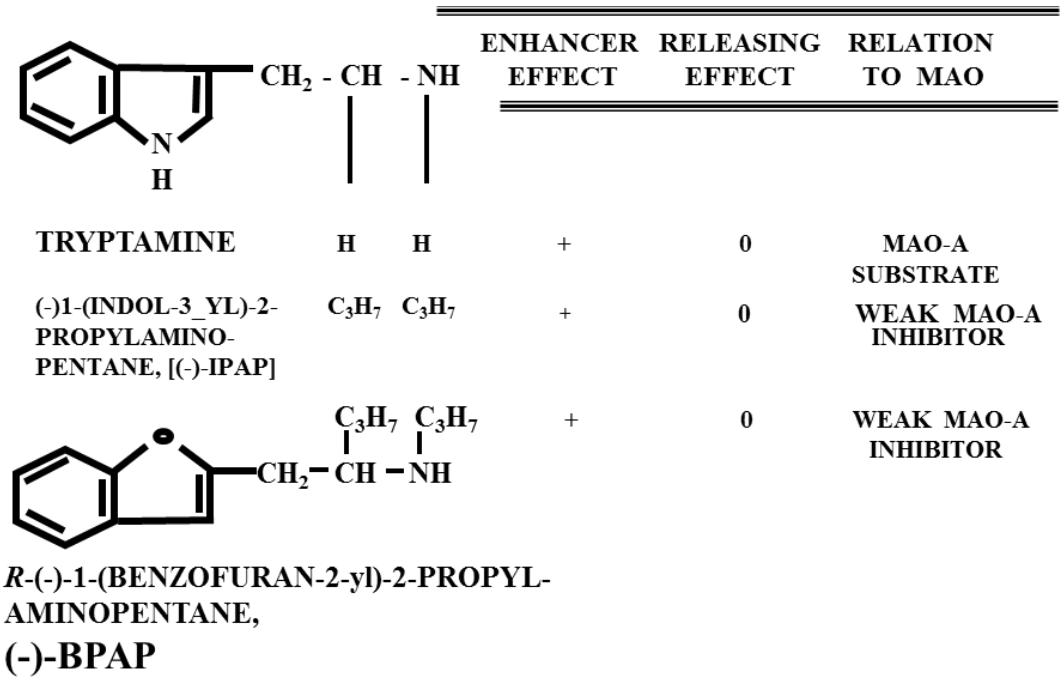


Fig.19. Chemical structure and pharmacological spectrum of tryptamine, the natural enhancer substance; (-)-IPAP, the simplest tryptamine-derived synthetic enhancer substance; and (-)-BPAP, the tryptamine-derived most representative enhancer substance.

Figure 20

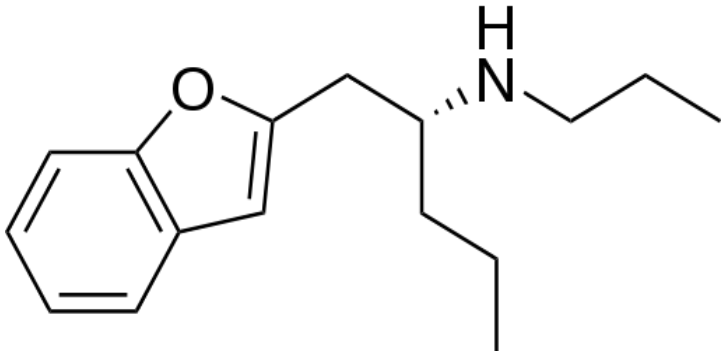
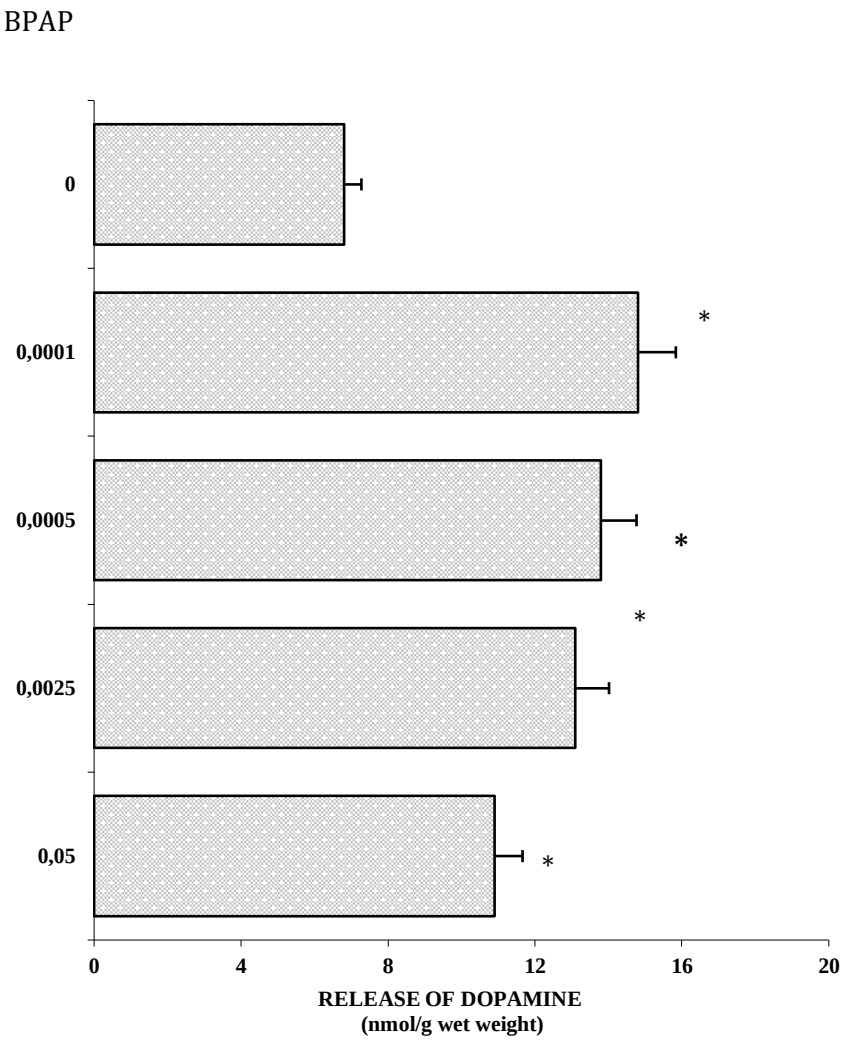


Fig.20. Systematic (IUPAC) name: (2*R*)-1-(1-benzofuran-2-yl)-*N*-propylpentane-2-amine and structure of BPAP, the presently known most potent synthetic enhancer substance, selected as an experimental tool for detecting hitherto unknown enhancer-regulations in the mammalian brain.

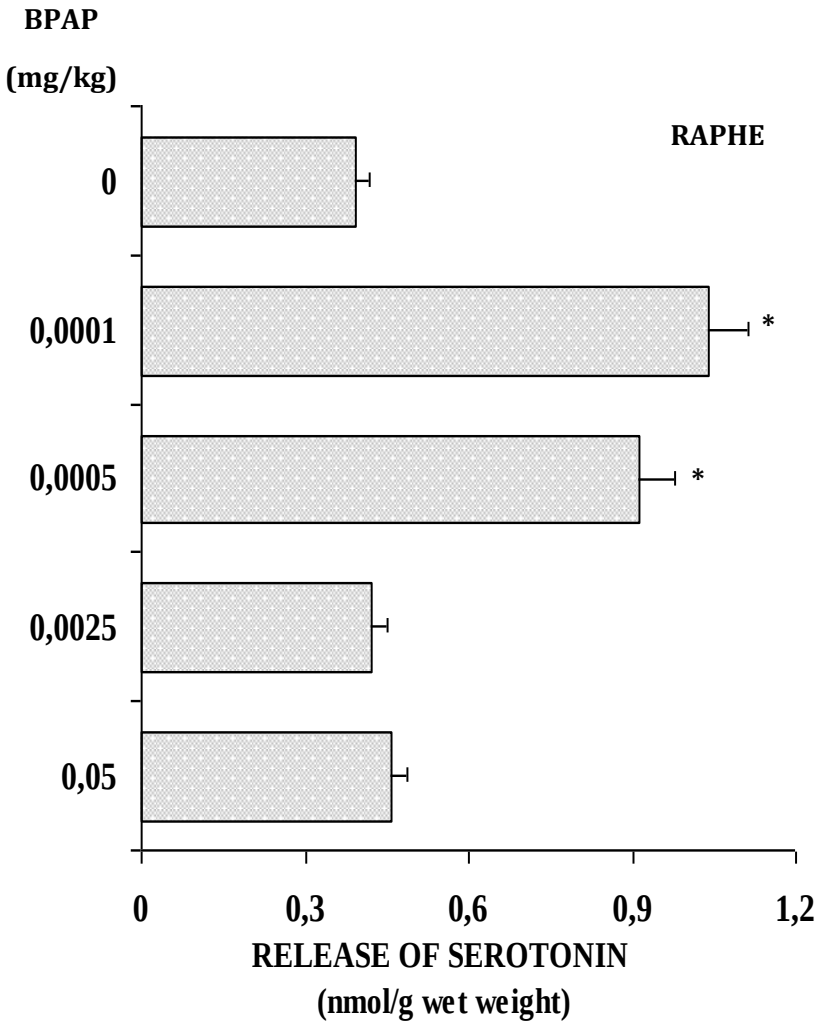
Figure 21



Paired Student's *t*-test. **P*<0.01

Fig.21. Illustration of the significant enhancement of the outflow of dopamine from the raphe of rats isolated 30 min after the subcutaneous administration of a single dose of BPAP. The amount of dopamine emitted from the tissue within 20 min following the administration of different doses of BPAP was measured according to Knoll and Miklya (1995). Vertical lines show SEM.

Figure 22



Paired Student's *t*-test. **P*<0.01

Fig.22. Illustration of the significant enhancement of the outflow of serotonin from the raphe of rats isolated 30 min after the subcutaneous administration of a single dose of BPAP. The amount of serotonin emitted from the tissue within 20 min following the administration of different doses of BPAP was measured according to Knoll and Miklya (1995). Vertical lines show SEM.

BPAP enhances the outflow of dopamine from the substantia nigra of rats in a subcutaneous dose of 0.0001 mg/kg (Fig.21); and in contrast to DEP, which is practically

ineffective on the serotonergic neurons; BPAP is a highly potent enhancer of the serotonergic neurons (Fig.22). *Note the peculiar dose dependency characteristic to the specific enhancer effect of BPAP: 0.0001 mg/kg is the peak dose; 0.0025 mg/kg is ineffective.*

To date, BPAP is the most selective and potent experimental tool to investigate the enhancer regulation in the catecholaminergic and serotonergic neurons of the brain stem. The enhancer effect can be detected following the subcutaneous administration of low amounts of BPAP (Table 10), as well as following the addition of the substance into the organ bath of freshly isolated discrete brain areas (Table 11).

Table 10

Series of experiments	Dose mg kg^{-1}	Amount of biogenic amine (nmol g^{-1} wet weight) released from the tissue within 20 min				
		Striatum	Substantia nigra	Tuberculum olfactorium	Locus coeruleus	Raphe
		dopamine			*norepinephrine*	*serotonin*
Single dose treatment. Measurement 30 min after the sc. injection of the compound						
Saline	-	4.5±0.15	6.8±0.18	4.9±0.15	4.7±0.10	0.391±0.02
BPAP	0.0001	4.7±0.14	14.8±0.36****	7.2±0.23****	6.6±0.10***	1.040±0.03***
	0.0005	4.8±0.16	13.8±0.23****	6.7±0.08****	15.4±0.55****	0.914±0.03***
	0.0025	5.7±0.19***	13.1±0.21****	6.9±0.31****	3.9±0.05**	0.421±0.03
	0.0500	6.5±0.09****	10.9±0.11****	7.7±0.19****	4.3±0.25	0.457±0.01
Treatment for three weeks, once daily. Measurement 24 hours after the last sc. injection						
Saline	-	3.8±0.18	5.8±0.18	4.4±0.24	3.9±0.10	0.403±0.01
BPAP	0.0001	4.4±0.12*	8.8±0.28****	4.6±0.17	7.4±0.15***	0.870±0.02***
	0.0005	3.9±0.18	8.3±0.23	4.2±0.23	4.1±0.05	1.907±0.04****
	0.0025	5.5±0.05****	8.7±0.34****	6.0±0.30***	3.7±0.1	0.212±0.03***

0.0500	4.8±0.12***	9.4±0.13****	6.3±0.09****	4.1±0.40	0.136±0.01***

*P<0.05 **P<0.02 ***P<0.01 ****P<0.001

Table 10. Release of catecholamines and serotonin from selected discrete brain regions of male rats treated with BPAP.

Table 11

		Amount of biogenic amine (nmol g^{-1} wet weight) released from the tissue within 20 min				
		Striatum	Substantia nigra	Tuberculum olfactorium	Locus coeruleus	Raphe
Series of experiments	Concent-ration (M)	*dopamine*			*norepinephrine*	*serotonin*

Saline	-	3.0±0.06	6.1±0.32	3.2±0.07	4.0±0.15	0.364±0.01
BPAP	10^{-4}	3.4±0.19*	7.6±0.29**	4.1±0.26**	4.5±0.50	0.473±0.02
	10^{-8}	3.9±0.25**	9.9±0.35****	4.6±0.11****	5.4±0.05**	0.547±0.03***
	10^{-12}	4.3±0.19****	9.7±0.21****	4.4±0.10****	5.5±0.05**	1.716±0.02****
Saline		3.4±0.08	5.5±0.05	3.6±0.11	4.2±0.15	0.359±0.01
BPAP	10^{-5}	3.8±0.17	9.0±0.19****	4.6±0.20***	5.0±0.35	0.501±0.01**
	10^{-9}	4.9±0.13****	8.2±0.19****	5.1±0.13****	4.7±0.05	0.878±0.05***
	10^{-14}	4.4±0.23***	9.5±0.30****	5.0±0.27***	7.4±0.25***	0.454±0.05
Saline		3.0±0.22	6.1±0.29	3.3±0.22	4.4±0.15	0.351±0.03
BPAP	10^{-6}	3.7±0.19	9.5±0.17****	3.8±0.11	9.3±0.20***	0.910±0.03***
	10^{-10}	5.0±0.13****	9.4±0.25****	5.0±0.22***	4.6±0.25	1.462±0.07***
		5.9±0.28****	15.1±0.38****	6.1±0.22****	8.2±0.35***	0.913±0.02***

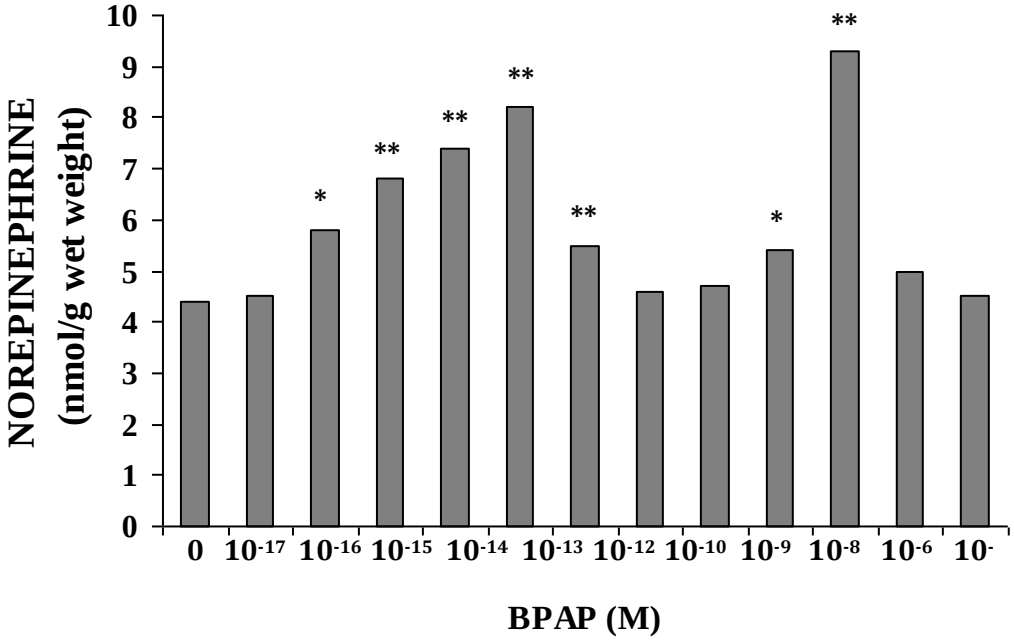
10^{-13}

*P<0.05 **P<0.02 ***P<0.01 ****P<0.00

Table 11. *Ex vivo* effect of BPAP on the release of catecholamines and serotonin from selected discrete brain regions of male rats.

2.3 Demonstration that (-)-BPAP on rats exerts its enhancer effect *a.* on the isolated locus coeruleus and *b.* on learning performance in the shuttle box with the same peculiar bi-modal, bell-shaped concentration effect curve, characteristic for the enhancer substances. Fig.23 shows the dose-dependency of the enhancer effect of BPAP on isolated locus coerulei of rats. Like in Fig 12 on shuttle box experiments with DEP, we see here with BPAP two characteristic bell-shaped concentration/effect curves. According to Fig.23, BPAP exerts on the noradrenergic neurons its specific enhancer effect with a peak at 10^{-13}M concentration, and its non-specific enhancer effect with a peak at 10^{-6}M concentration (see Knoll et al. 2002, for details).

Figure 23



*P<0.01, **P<0.001

Fig.23. Bi-modal, bell-shaped concentration effect curve characteristic to the enhancer effect of BPAP on isolated locus coerulei of rats. BPAP was given to the organ bath of the quickly removed locus coerulei. Eight organs were used for the analysis of each concentration. The amount of norepinephrine emitted within 20 minutes from the tissue in the presence of different concentrations of BPAP was measured according to Knoll and Miklya (1995). Paired Student's *t*-test.

We have found the same dose-effect relation regarding the specific enhancer effect of BPAP in *in vivo* experiments. We treated rats with saline or a single dose of 0.05, 0.0025, 0.0005, and 0.0001 mg/kg BPAP, respectively. Thirty minutes after the subcutaneous injection, we quickly removed the locus coeruleus and measured, (using the method of Knoll and Miklya, 1995), the amount of NE released from the tissue within 20 minutes. In this experiment, the most effective dose of BPAP, 0.0005 mg/kg, increased the outflow of NE from 4.7±0.10 nM/g (control) to 15.4±0.55 nM/g ($P<0.001$), but a 100-times higher dose of BPAP (0.05 mg/kg) did not change it (4.3±0.25 nM/g) (Knoll et al. 2002).

In Fig.12 the dose-response curve of the enhancer effect of DEP measured in the shuttle box already demonstrated that enhancer substances stimulate the enhancer-sensitive neurons in the brain stem in a peculiar manner and the same experiment with BPAP (see in Part 2.5 Fig. 26) shows the same bi-polar, bell shaped dose-effect curve.

2.4. The concept that the peculiar dose-dependency of natural enhancer substances might be responsible for the individual differences in behavioral performances. In our behavioral studies on rats we continuously observed the great individual variation in sexual activity and learning performance in any random population of mammals of the same strain. To illustrate such extreme individual differences in a random rat population: in our second longevity study (Knoll et al. 1994) we selected from a population of sexually inexperienced 1600 Wistar-Logan rats the ones with the lowest sexual potency and found 94 rats which did not display in four consecutive weekly mating tests any sign of sexual activity. These “non-copulators” remained inactive until they died (low performing /LP rats). On the other hand, we found 99 males which displayed at least one ejaculation in each of the four tests (high performing/HP rats).

The discovery of the bell-shaped concentration/effect curve of the enhancer substances, in the low nano-molar concentration range, offers the first reasonable explanation for the great individual variation in behavioral performances. Since an *optimum* concentration of the enhancer substance was needed for the *optimum* performance, *I postulate that the substantial individual differences in behavioral performances are due to the peculiar dose-dependency of the endogenous enhancer substances.*

This approach offered us a new perspective on the results of our two longitudinal studies on rats (first longevity study: Knoll 1988; Knoll et al. 1989; second longevity study; Knoll et al. 1994). Details of these longevity studies were shown in Part 1.

Considering the unique dose-related effect of an enhancer substance, we assume that out of the 1600 rats, the 99 HP rats produced their endogenous enhancer substances responsible for sexual performance at the peak of the bell-shaped concentration/effect curve, while the 94 LP rats produced them at the least active part of the curve. The overwhelming majority of the population (1407 rats) fell in between these two extremes.

An analysis of the ability of rats to acquire the glass-cylinder-seeking drive is another example that convincingly illustrates the great individual differences in the behavioral performances of rat (see Sect.1.3 and 4.2. in Knoll 2005). As mentioned earlier we observed that only two rats out of a 100 maintained the acquired glass-cylinder-seeking function with unchanged intensity throughout their life. Presumably the specific endogenous enhancer substances in the cortical neurons responsible for the operation of the glass-cylinder-seeking drive were mobilized in these two rats in the optimum concentration. Thus, we may look upon these two rats as the most talented in the tested population regarding the measured function.

All in all, the discovery that PEA is a natural enhancer of the catecholaminergic and serotonergic neurons in the brain stem, and the fact that we successfully fabricated a much more potent and selective enhancer substance than DEP, is a heavy argument for the thesis that enhancer regulation operates in the catecholaminergic and serotonergic neurons in the brain and places at our disposal tools through which we can maintain the activity of enhancer-sensitive cells on higher activity level without changing their physiological milieu (see Knoll 2012, Chapter 9).

As briefly analyzed in Part 1, the enhanced activity of the catecholaminergic brain engine from weaning until full scale sexual maturity is regarded primarily responsible for the most delightful phase of life, the glorious uphill journey. Sexual hormones bring back the enhancer regulation in the catecholaminergic and the serotonergic neurons in the brain to the pre-weaning level, thus terminating developmental longevity. The post-developmental, downhill period of life starts and lasts until the “natural death”. Since aging of the catecholaminergic system in the brain plays a leading role in the natural, slow decay of physical and mental welfare, we need to start fighting against aging of the catecholaminergic brain engine as soon as sexual maturity is reached.

The dopaminergic machinery is the most rapidly aging neuronal system in our brain. The DA content of the human caudate nucleus decreases steeply, at a rate of 13% per decade over age 45. We know that symptoms of PD appear if the DA content of the caudate sinks below 30% of the normal level. Experimental and clinical experiences show that daily dosages of DEP keep the brain engine's activity on a higher activity level in humans. From sexual maturity, a low daily dose of DEP (1 mg) is sufficient to significantly slow the pace of the aging-related decay of the dopaminergic neurons. Even if we assume only a small protective effect of DEP in healthy humans against the age-related decrease in striatal dopamine, for example from 13% per decade to 10% per decade, this translates to a minimum 15-year extension in average lifespan and a considerable increase of the human technical lifespan (TLS_h), which is now estimated to be around 120 years (see Fig. 6 in Knoll 1992b).

We demonstrated in earlier longevity studies that male rats injected with DEP preserved their learning ability longer, lost their ability to ejaculate later, and lived longer than their placebo treated peers. Assuming that the selective inhibition of B-type MAO in the brain is responsible for these beneficial effects we performed two longevity studies with 0.25 mg/kg DEP, the dose which blocks completely MAO B activity in the brain (Knoll 1988; Knoll et al. 1989; 1994). The discovery that DEP is a PEA-derived synthetic CAE-substance, and the development of BPAP, the tryptamine-derived, more potent synthetic CAE-substance than DEP, devoid of MAO-B inhibitory potency, drew our attention to this new subject.

2.5. The first longevity study with BPAP. Experimental evidence that lifelong maintenance of rats on a low dose of BPAP (0.05 and 0.0001 mg/kg, respectively), significantly prolongs their life. As was shown earlier, a bi-modal, bell-shaped concentration effect curve is characteristic to the enhancer effect of both DEP (Fig.12) and BPAP (Fig.23). BPAP enhanced the activity of the noradrenergic neurons in the femto/picomolar concentration range ("specific enhancer effect"), and also in a 10 million times higher concentration range ("non-specific enhancer effect"). DEP is a less potent CAE-substance than BPAP, but otherwise it exerts its specific and non-specific enhancer effect with the same characteristics as BPAP (see Fig.12). The discovery of the CAE effect of DEP inspired the working hypothesis that this effect is responsible for the finding that rats treated for life three-times a week with 0.25 mg/kg DEP, (*which exerts its non-specific*

enhancer effect; see Fig.12), preserved their learning ability, ejaculating power; and they lived longer than their placebo treated peers. To substantiate the assumption we performed a longevity study, treating rats with the optimal dose of BPAP for its specific enhancer effect.

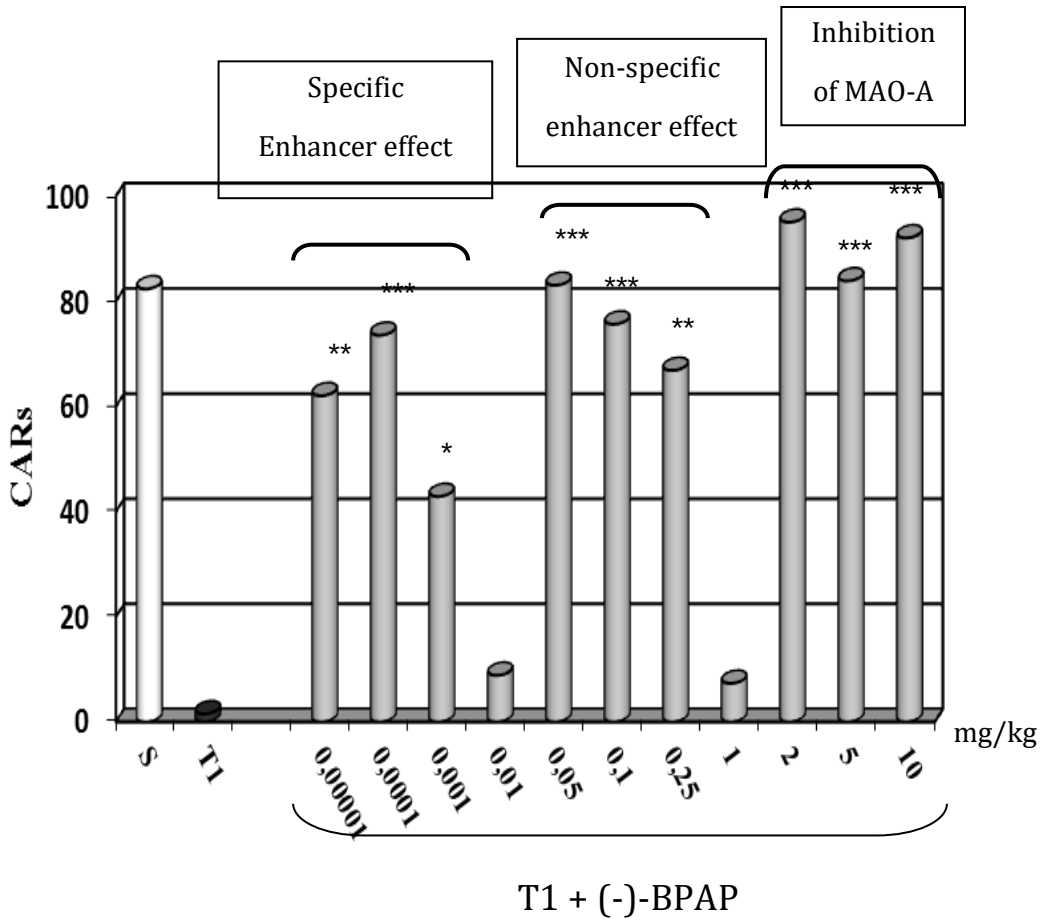
In a modified version of the shuttle box experiment, the acquisition of a two-way conditioned avoidance reflex (CAR) was analyzed during 5 consecutive days, as described in Part 1. Tetrabenazine-treatment (1 mg/kg sc.) causes a learning deficit which can be antagonized by the administration of a synthetic CAE substance or an A-type MAO inhibitor, whereas selective inhibition of B-type MAO or inhibition of the reuptake of catecholamines and/or serotonin is ineffective (Knoll et al. 1992a).

As was shown earlier (Fig.12 and Fig.23), a bi-modal, bell-shaped concentration effect curve is characteristic to the enhancer effect. BPAP enhanced the activity of the noradrenergic neurons in the femto/picomolar concentration range ("specific enhancer effect"), and also in a 10 million times higher concentration range ("non-specific enhancer effect"). DEP is a less potent CAE substance than BPAP, but otherwise it exerts its specific and non-specific enhancer effect with the same characteristics as BPAP (see Fig.12).

Fig.24 shows the dose-related effect of BPAP in the shuttle box.

For the longevity study we selected the optimal dose that elicited the specific (0.0001 mg/kg) and the non-specific (0.05 mg/kg) enhancer effect. Since BPAP blocks the activity of MAO-A in higher than 2 mg/kg dose (Knoll et al. 1999), it antagonized tetrabenazine-induced learning deficit also in the extremely high dose-range (2-10 mg/kg).

Figure 24



*P<0.01; **P<0.001

Fig.24. *Selection of optimal doses of BPAP for the longevity study in the shuttle box.* Measured: (S) the ability of saline-treated (control) rats to fix conditioned avoidance responses (CARs); (T1) the inhibition of the learning ability of rats treated subcutaneously with 1 mg/kg tetrabenazine, one hour prior to training; [T1 + BPAP] the ability of BPAP to antagonize in a dose related manner the inhibitory effect of tetrabenazine. Significance in the performance between the groups was evaluated by multifactor analysis of variance (ANOVA).

Figure 25

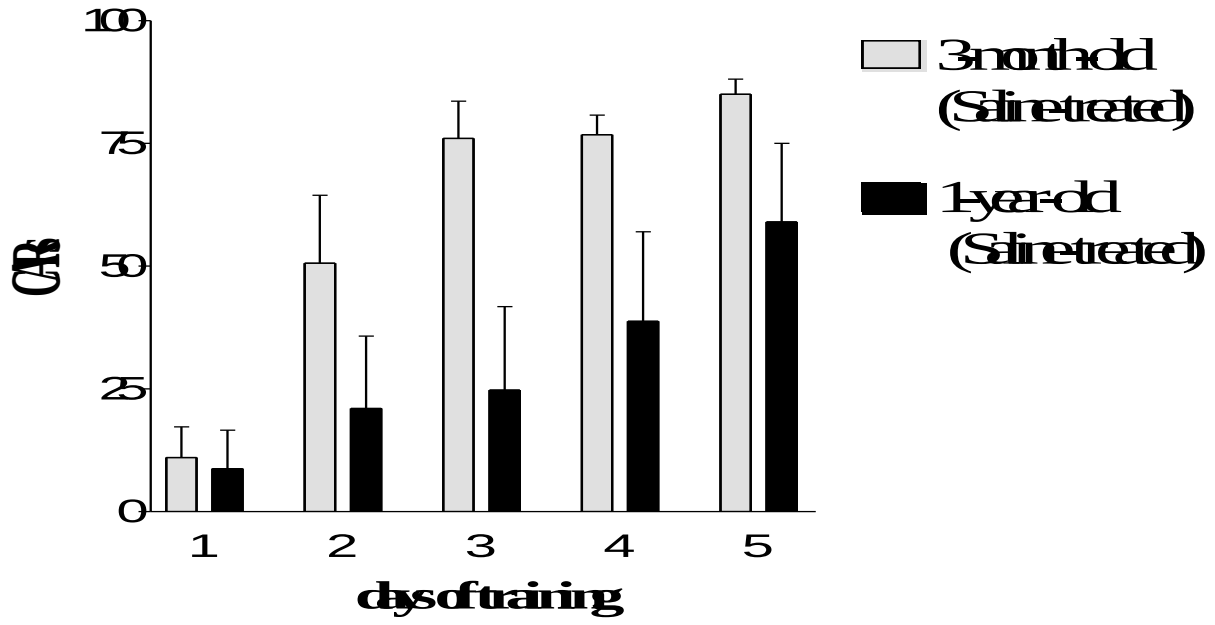


Fig.25. Experimental evidence that 3-month-old rats are significantly better learners than their 1-year-old peers *(P*<0.001). Significance in the performance between the groups was evaluated by multi-factor analysis of variance (ANOVA). Rats were trained in the shuttle box with 100 trials per day. Conditioned avoidance responses (CARs).

Fig.25 shows that, due primarily to normal aging of the catecholaminergic neuronal system in the brain stem, saline-treated 3-month-old rats are significantly better performing than their 1-year-old peers. On the other hand, Fig.26 shows that due to the anti-aging effect of the 0.0001 mg/kg dose of BPAP, one year old rats treated for 10 months subcutaneously, 3 times a week, with this extremely low dose of BPAP, were significantly better learners in the shuttle box than their saline-treated peers, indicating that the specific enhancer effect of BPAP is responsible for the anti-aging effect of the compound.

Figure 26

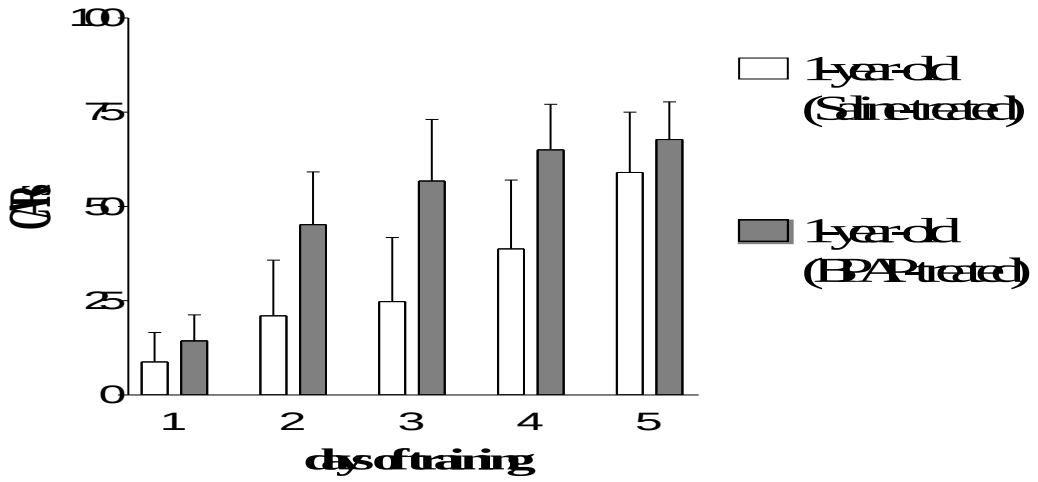


Fig.2 -year-old rats were anogram/kg!) and in the shuttle box actor analysis of variance (ANOVA). The BPAP treated rats performed significantly better than their saline-treated peers ($P<0.05$).

As was shown in Part 1, the enhancer regulation of the catecholaminergic and the serotonergic neurons in the brain starts working on a significantly higher activity level after weaning, and the intensified activity subsists until sexual maturity is reached; thereafter returns to the pre-weaning level. Developmental longevity, the uphill period of life, is the phase between weaning and sexual maturity. Sexual hormones dampen the intensified enhancer regulation in the catecholaminergic and serotonergic neurons in the brain. Activity returns to the pre-weaning level and this reflects the transition from adolescence to adulthood. As soon as sexual maturity is reached, the post-developmental phase, the downhill period of life, begins and lasts until natural death.

During the post-developmental period, the enhancer regulation in the catecholaminergic brain machinery is on a slow continuous decline. The catecholaminergic neurons play a key role in the efficiency of learning performances, drive motivated behavior, etc. The continuous decline of their activity over time plays a crucial role in the behavioral consequences of brain aging. With the daily preventive administration of a synthetic enhancer substance from sexual maturity until death, we can maintain the activity of the catecholaminergic and serotonergic neurons on a higher activity level, so we now have a new method to safely slow the aging-related decay of physical and mental functions (Knoll 2012).

In the past, when we performed our two longevity studies and worked with the robust

Wistar-Logan rats, we observed that the males which completed the second year of life never displayed a single ejaculation in the weekly mating test. We later experienced that the Sprague-Dawley CFY or Wistar (Charles-River) rats also lost this ability at this age. Our studies clarified that the aging-related irresistible decay of the dopaminergic brain machinery is responsible for this change. Saline-treated CFY male rats reached the stage of inability to ejaculate at 112±9 weeks, their DEP-treated peers reached that stage at 150±12 weeks (Knoll, 1993).

In our first longevity study performed on Wistar-Logan rats, we started to work with 132 sexually inexperienced 2-year old males, and we tested their sexual activity in four consecutive weekly mating tests during 24 months. The rats were divided into three groups: 46 "non-copulators", 42 "mounting" rats and 44 "sluggish" rats (displaying mountings and intromissions). Thereafter, we treated 66 rats with saline and 66 rats with 0.25 mg/kg DEP, three times a week, and observed their behavioral performances to the end of their life.

The saline-treated group of the "non-copulators" died out first; the "mounting" rats lived longer; the longest living rats were in the "sluggish" group (see Table VI in Knoll 1988). DEP treatment prolonged the life in each group significantly. The 66 salt-treated rats lived in average 147.05± 0.56 weeks; the 66 DEP-treated rats lived in average 197.98± 2.31 weeks.

The fact that the saline-treated "non-copulators" died out first and the finding that DEP, the specific stimulant of the catecholaminergic brain stem neurons, keeps the rats on a higher activity level and prolongs their life, suggested that the catecholaminergic engine of the brain, which is of crucial importance in activating the cortex, is responsible for the lifespan-prolonging effect. Thus, the brain engine works in the 2-year old "non-copulator" males on a lower activity level than in the 2-year old "sluggish" males. This working hypothesis, based on the results of the first longevity study, determined the planning of the second longevity study. *We decided to start the experiment with younger, 18-month-old rats. We selected from a huge population of Wistar-Logan males (1600) the "non-copulators" (94 low performing, LP rats) and the sexually most active ones (99 high performing, HP rats), measured their sexual potency and learning ability until the end of their life, and treated them with saline and DEP, respectively.* The results of this longevity study, shown in Fig.2, were already discussed in Part 1.1.

The outcome of the first longevity study performed with the low doses of DEP as shown in Fig.14 and 15 points at the role of the enhancer effect in the DEP-treatment-induced

prolongation of life, though this effect remained below the statistically significant level. Since BPAP is the most selective and potent enhancer substance presently available, we hoped that the outcome of the longevity study performed with 0.05 and 0.0001 mg/kg BPAP, respectively, might give the final answer regarding the role of the enhancer effect in the prolongation of mammalian life.

Fig. 27 and 28 shows that the groups of rats treated with 0.05 and 0.0001 mg/kg BPAP, respectively, 3-times a week from their second month of age until death, lived significantly longer than their saline-treated peers. To further support the conclusion that the enhancer effect of DEP and BPAP are responsible for the prolongation of mammalian life, a longevity study treating rats *daily* with the same low doses of DEP and BPAP is currently in progress.

Figure 27

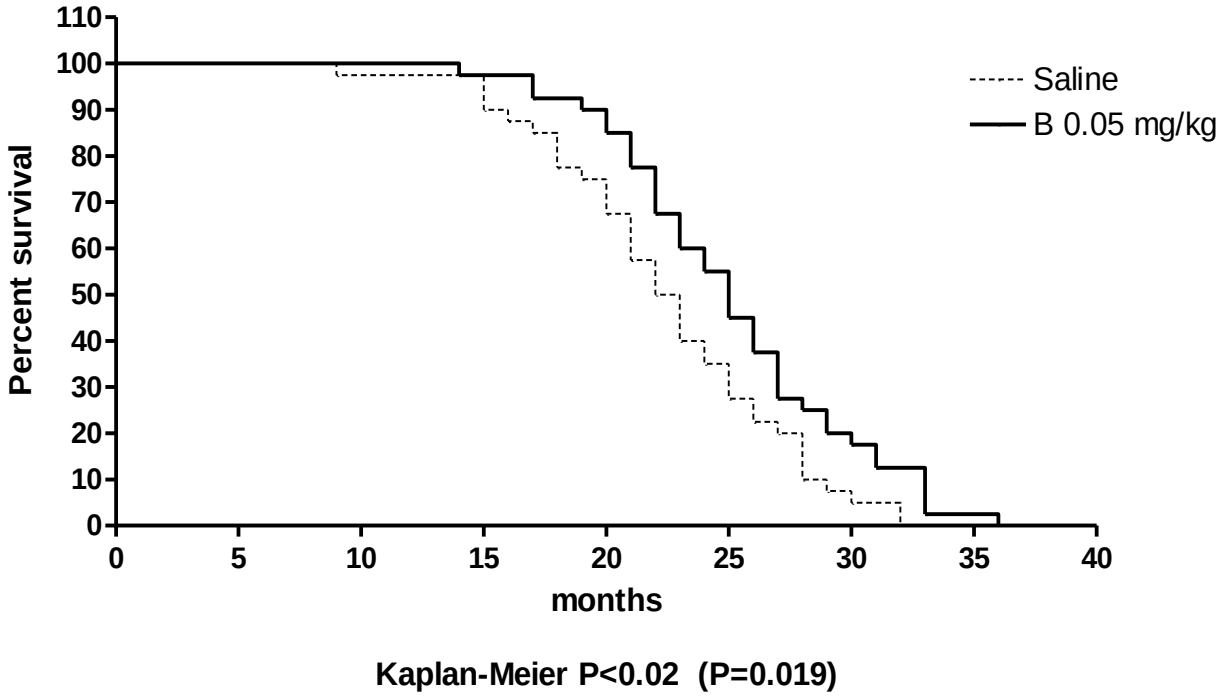


Fig. 27. The difference in the course of changes in survival between groups of rats (N=40) treated with saline and 0.05 mg/kg BPAP, respectively.

Figure 28

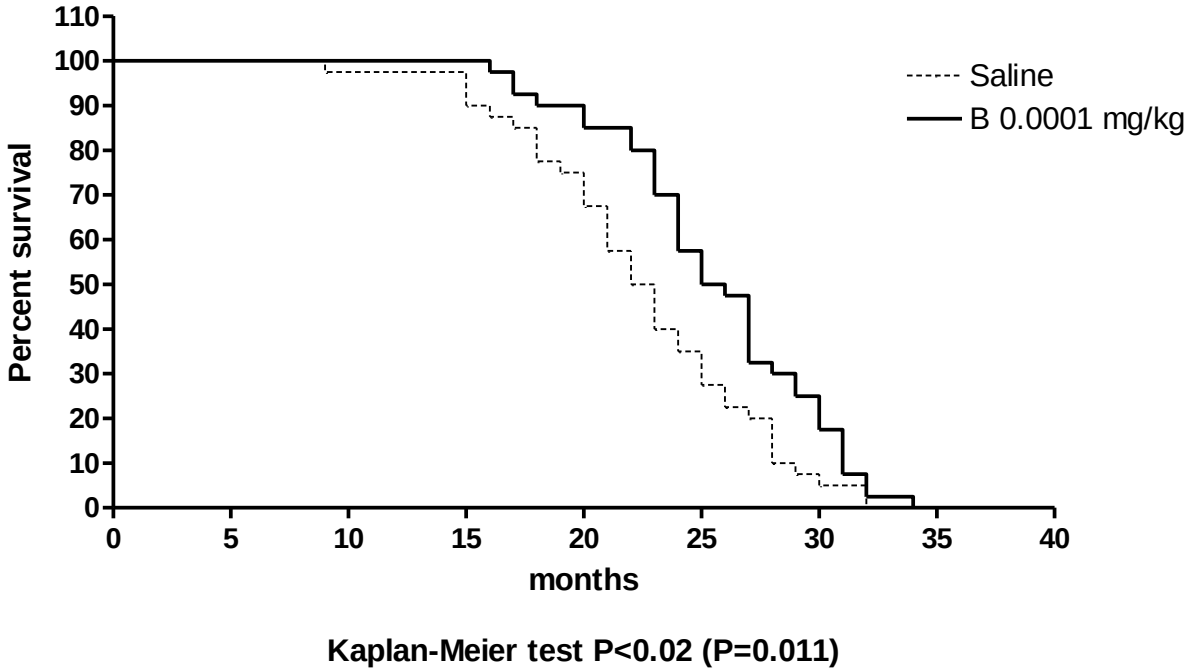


Fig.28. The difference in the course of changes in survival between groups of rats (N=40) treated with saline and 0.0001 mg/kg BPAP, respectively.

Table10

TREATMENT	AVERAGE LIFESPAN IN WEEKS		
Saline	94.23 ± 3.48		[100%]
BPAP 0.05 mg/kg	107.00 ± 3.45	(P<0.02)	[114%]
BPAP 0.0001 mg/kg	107.00 ± 3.14	(P<0.01)	[114%]

Table10. The average lifespan of rats in weeks, treated with 0.05 and 0.0001 mg/kg BPAP, respectively.

Table 11

TREATMENT	SHORTEST LIVING RAT (weeks)	LONGEST LIVING RAT (weeks)
Saline	36 [100%]	135 [100%]
BPAP 0.05 mg/kg	59 [164%]	153 [113%]
BPAP 0.0001 mg/kg	65 [180%]	145 [107%

Table 11. The shortest and longest living rat in the groups treated with saline and BPAP, respectively.

Table 10. shows the average lifespan of rats treated with saline, 0.05 and 0.0001 mg/kg BPAP, respectively. The difference in the average lifespan between saline and both 0.05 and 0.0001 mg/kg BPAP-treated rats was statistically significant ($P<0.02$ and $P<0.01$, respectively).

Table 11. shows the shortest and longest living rats in the groups treated with saline, 0.05 and 0.0001 mg/kg BPAP, respectively. The shortest living rats lived in the 0.0001 mg/kg BPAP-treated group with 65 weeks longer and in the 0.05 mg/kg treated rats with 59 weeks longer than the shortest living rat in the saline-treated group. The longest living rat in the 0.0001 mg/kg treated group lived 10 weeks longer and in the 0.05 mg/kg treated group lived 18 weeks longer than the longest living rat in the saline-treated group.

The data shown in Fig. 27-28 and in Tables 10 and 11 furnish unequivocal experimental evidence that the enhancer effect of BPAP is responsible for the significant prolongation of life.

Closing Remarks

The discovery that catecholaminergic and serotonergic neurons are enhancer sensitive entities; the realization that PEA and tryptamine are natural enhancers of these neurons; the development of their synthetic varieties, DEP, PPAP, and BPAP, all furnish sufficient evidence that there is a reasonable chance to develop a safe preventive medication to improve the quality and prolong the duration of human life.

Lifelong preventive medication requires unique drug safeness. Due to their peculiar mechanism of action and safety margin, only the synthetic enhancer substances meet this requirement. *BPAP on rats exerts its **specific** enhancer effect in a subcutaneous dose as low as **0.0001 mg/kg** and even **20 mg/kg** is tolerated without any sign of toxic effects.* This is truly an exceptional safety margin.

The discovery of the essential changes in the enhancer-regulation of the catecholaminergic and serotonergic neurons during the lifetime of rats, and the peculiar mechanism of action of enhancer substances deserve distinct consideration.

The enhancer-sensitive catecholaminergic and serotonergic neurons work before weaning at a low, "economic" level, which is dramatically intensified after weaning. The tense excitement remains unchanged during the developmental phase of life, from weaning until sexual maturity (Part 1.6). Sexual hormones (estrone, testosterone) return the enhanced catecholaminergic and serotonergic activity to the pre-weaning, "economy" level, terminating the developmental phase of life. This change is also the beginning of the slow, continuous decay of the enhancer regulation (aging) until "natural death". It is obvious that only the development of a safe and efficient preventive pharmacological intervention, starting immediately after the completion of sexual maturity, can significantly slow brain aging (Part 1.7).

In the extremely low dose range in which they exert their specific enhancer effect, the enhancer substances selectively transform the lower performing enhancer sensitive neurons into better performing ones.

In retrospection the outcome of the second longevity study, published in 1994, was the ***first*** undeniable proof of this mechanism. As shown in Part 1, we selected out of 1600, 28-week-old

males of the robust, long-living Wistar-Logan strain of rats, the 94 sexually lowest performing (LP) and 99 sexually highest performing (HP) ones, and treated them with saline and DEP, respectively, for life. *The saline treated LP rats (n=44) lived 134.58 ± 2.29 weeks, and their HP-peers lived 151.24 ± 1.36 weeks (P<0.001).* The DEP treated LP rats (n=49) lived significantly longer than their saline treated peers and lived as long as the saline-treated HP rats. DEP treatment also transformed the innate HP rats (n=50) into better performing ones. ***They lived 185.30 ± 1.96 weeks. Out of the 50 rats, 17 lived longer than the maximum lifespan ever observed during a long observation period on hundreds of untreated or saline treated rats in the strain used in our studies.***

Already the results of the first longevity study (Knoll, 1988) fascinated me so greatly that I decided to undertake a self-experiment and began taking 1 mg of DEP daily on January 1, 1989 at the age of 64. I am now 90 years old and my self-experiment augurs so far well.

Since a bi-modal, bell-shaped concentration effect curve is characteristic to the enhancer effect, an optimum concentration of the enhancer substance was needed for an optimum performance. Both lower and higher concentrations were less effective, so the reasonable working hypothesis is that the substantial individual differences in behavioral performances are due to a peculiar dose dependency of the endogenous enhancer substances. According to this concept in the afore-mentioned second longevity study the enhancer sensitive neurons responsible for the measured performance produced their endogenous enhancer substance in the 99 HP rats at the peak of the bell-shaped concentration-effect curve, while the 94 LP rats produced them at the least active part of the curve with the overwhelming majority of the population (1407 rats) scattered between these two extremes.

All drugs used today harshly change the physiological milieu of highly sophisticated living material in their pharmacologically effective dose, so they are, in principle, unsuitable for lifelong daily administration. If a drug is long-term prescribed as a daily protective agent for some well-established reason, like aspirin, for example, only a fraction of the usual daily dose is given.

The enhancer substances represent a case without any parallel. In the low concentration in which they exert their specific enhancer effect, they transform the lower performing enhancer sensitive neurons into better performing ones, thus leaving the physiological milieu of the neurons unchanged (Knoll, 2012 Chapter 9). As shown in this essay (Part 2.5), BPAP significantly

prolonged the lifespan of rats treated *only three times a week* with the incredibly low, 0.0001 mg/kg dose, in which the compound exerts the peak of its specific enhancer effect.

Since DEP (Selegiline), is the only synthetic CAE substance in clinical use worldwide, it is reasonable to suggest a daily 1 mg dose to serve as a preventive agent from sexual maturity to slow the aging of the catecholaminergic brain engine. As repeatedly demonstrated, DEP is a perfectly safe option for this purpose. Nevertheless, BPAP, the therapeutic efficiency of which still needs establishing, overshadows the potency of DEP. *BPAP, the highly potent and selective synthetic enhancer substance, is an ideal experimental tool for detecting unknown enhancer-sensitive brain regulations*. Since our knowledge regarding the enhancer regulation is in its infancy, we see just the peak of the iceberg. The prospects of revealing by the aid of BPAP unexplored enhancer regulations in the mammalian brain are quite promising. Experiments in progress are in harmony with these expectations.

All in all, the discovery of the enhancer regulation in the mammalian brain; the already known unique pharmacological spectrum of DEP and BPAP; the aging-related decline of the enhancer-sensitive neurons; and ***the finding that lifelong treatment of rats with 0.0001 mg/kg BPAP significantly prolonged life, is*** *groundbreaking evidence for a safe and effective preventive medication.*

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February 4, 2016

Hector Warnes' comment

I don't really feel qualified to dissect Professor Knoll's great contribution. He is a basic scientist with findings in animal and human research which support his views. I am a clinician with interest in this particular area of research.

I am glad that Professor Knoll has addressed three important issues in his "history of enhancer regulation":

1. Innate versus acquired drives.
2. L-Deprenyl as an antidepressant, anti-Parkinson and anti-aging agent.
3. Theories that would be compatible with the anti-aging effect of L-Deprenyl.

1. Innate versus acquired drives

I would like to start my comments by citing Konrad Lorenz: "Instinctively innate and individually-acquired links often succeed each other directly in the functionally uniform action chains of higher animals. I have termed this phenomenon 'instinct training interlocking' and emphasized that similar interlocking also occurs between instinctive and insightful behavior" (Lorenz 1957, p. 137). Further, Lorenz asserts that complex behavior patterns in higher animals and men, which are built on an instinctive basis but are imbued with learned or acquired components, would be considered an example of interlocking. Professor Knoll suggests that innate drives are released by the mesencephalic- and acquired drives by the telencephalic- brain. I would be interested to know how he separates drives from instincts.

Professor Knoll has experienced in his own flesh and mind the atrocities committed by the Nazis. Like any totalitarian regime, the Nazis shared a high degree of fanaticism which they displayed with a vengeance and readiness to respond to specific stimuli by invoking a combination of numerous interlocking functions (innate releasing mechanism). Freud (1921) thought that the group mind often obliterates an individual's acquired values. Once the individual is united with the group, it unleashes in him a frenzy of enthusiasm and his most blunt and primitive side. In that collective mentality, one may acquire a feeling of invincible power while

caught in the discourse with the leader in a unique altered state of consciousness. The action of the masses transforms and drives (contagion) and under this action one becomes pray of great suggestibility. The mutual tie between members of a group is in the nature of identification with the leader.

Professor Knoll, himself a survivor of the Holocaust, rose to greatness as a scientist in spite of the severe traumas which he endured, including persecution, torture, hunger and loss of most of his family in concentration camps (Lager). He is living example of resilience and innate capacity to overcome (*überwinden*) the most traumatic experiences that a man can endure (Knoll 2005).

2. L-Deprenyl as an antidepressant, anti-Parkinson and anti-aging agent

On March 13, 2014 Miklya Ildiko (2014) wrote an outstanding review on Selegiline that was posted on the INHN website. 'Deprenyl or selegiline was developed by Professor Knoll and his team at Semmelweis University in Budapest, Hungary. It was patented in 1962 and its levo-rotatory enantiomer compound was discovered in 1967.

L-Deprenyl is an irreversible inhibitor of MAO-B. It has dopaminergic and catecholaminergic effects and unlike the MAO-A inhibitors it does not produce the 'cheese effect' (tyramine). However, in doses over 20 mg l-Deprenyl loses its selectivity and inhibits both the MAO-A and MAO-B enzymes. In a dosage of 5mg l-Deprenyl inhibits the MAO-B enzyme by 90%.

It was shown that l-Deprenyl was useful in the treatment of Parkinson's disease. In one study, by its dopaminergic effect it delayed the need to introduce L-Dopa treatment by 13 months. The adverse side effects of Selegiline in low doses are nausea and insomnia.

There has been an underutilization of L-deprenyl by clinicians perhaps for fear of side effects and length of 'wash out' time needed in case of emergency procedures. Until the early years of the 21st century, Selegiline in the doses from 5mg to 10mg per day orally was not found particularly effective in the treatment of major depressive disorders but useful in some cases of atypical depression and dysthymic disorders.

There was an early optimism followed by disappointment about its use as an antidepressant about 15 years ago. J. Alexander Bodkin at the McLean Hospital used Selegine as a transdermal patch that was approved by the US Food and Drug Administration for the treatment of depression, in 2006. Bodkin treated 177 depressed patients with the transdermal patch; 89 wore

patches with the active compound and 87 wore patches with no drugs. After 6 weeks, those patients who wore patches with the active compound had a remission rate of 42 % (37 patients). However, these significant results were not replicated in other clinical research (Cromie. 2002). In so far as I am aware, there was no comparative study of l-Deprenyl's efficacy in the treatment of depressive disorders with established antidepressant compounds, such as desvenlafaxine, mirtazapine, duloxetine, fluoxetine, agomelatin, bupropion and so on. Bupropion has a metabolite closely related to phenethylamine, an endogenous amphetamine.

The transdermal preparation acts almost exclusively in the brain. By passing hepatic metabolism and the elimination of the drug via the gut, it offers advantages in the treatment of some depressive disorders (Stahl 2011).

The original research of Professor Knoll on aging with l-Deprenyl showed a doubling of lifespan in Wister-Logan rats. When other investigators tried to replicate the study, they did not obtain the same results when using different strains of rats. This difference indicates that in longevity, genetic mechanisms are involved.

It is reasonable to assume that in the future it will become possible to determine by positron emission tomography (PET) the functional lesion in the nigrostriatal catecholaminergic neurons, the subclinical onset of Parkinson's disease and to delay the appearance of its clinical manifestations by the administration of l-Deprenyl. Perhaps the same will be the case for Alzheimer's disease when biological markers (amyloid plaques, apolipoproteins and protein Tau plus imaging) will become available for detecting high risk asymptomatic patients.

Peter Franz Riederer and his team in Vienna were one of the first researchers in 1975 who tried to replicate Professor Knoll's findings (Gerlach,Youdim, Riederer 1996). Professor. Riederer's findings were supportive of Professor Knoll's view that selegiline has neuroprotective activity.

3. Theories that would be compatible with the antiaging effect of L-Deprenyl

The first longevity study of Professor Knoll with l-Deprenyl, was published in 1988. Knoll considers phenylethylamine and tryptamine "natural enhancers" which delay theonset of brain decline. Several decades after the discovery of l-deprenyl, Knoll discovered another enhancer substance, R-1-benzofuran-2-yl-2-propylaminopentane (-)-or BPAP.

With aging, there are signs of entropy which lead to irreversible changes and eventually to death. Biological organisms show spontaneous fluctuations and by losing homeostasis become highly unstable dynamical systems. The changes which are taking place from conception to death include mutations of mitochondrial DNA with gradual accumulation of cells which are bio-energetically deficient. Mitochondrial dysfunction with the over-production of free radicals are paramount in the aging process. Our natural genetically determined life span might be altered also by epigenetic factors including the accumulation of molecular damage caused by the production of metabolically and environmental triggered free radicals, chromosomal mutations and nutritional factors. Obviously, the wear and tear of life itself plays a very important role in aging.

It would be interesting to test the 'enhancer' effect of l-Deprenylon the length of the telomeres (TTAGG hexanucleotides). The function of the telomeres is the protection of chromosomal deoxyribonucleic acid (DNA) from alterations. The length of telomeres is associated with the biological clock. We lose about 41 pairs per year. (Those Down's syndrome patients lose 133 pairs per year). Telomerase protects the chromosomal length by nurturing it with nucleotides. It is possible to determine whether one has a gradual decline of telomerase or a speedy decline. In case of "speedy decline", 'enhancer treatment' (that increases neurotransmitter activity) might reverse the process.

It was shown that l-Deprenyl like other antidepressants increase the Brain Derived Neurotrophic Factor and or the Nerve Growth Factor which act by their effect on neural plasticity-neurogenesis (like other antidepressants and antioxidants) hindering apoptosis and cellular death.

There is plenty of evidence that Delta sleep decreases gradually with age accompanying a decrease of growth hormone production and immunity. With immuno-incompetence there is an increase of infections, auto-immune disorders and cancer. During Delta sleep the brain uses less energy and restores its supply of adenosine triphosphate (ATP) (Besedowsky, Lange, Born 2012).

I must congratulate Prof. Knoll for his life work that has provided a new path to slow aging to the inevitable end.

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May 19, 2016

Joseph Knoll's reply to Hector Warnes' comment

Thank you Dr. Warnes for your thought-provoking comments.

Reply to "Innate versus acquired drives"

You ask me, how I separate drives from instincts. I never use the terms 'instinct, instinctive'. Mammalian behavior is fully comprehensible via the careful analysis of the operation of *inner drives in the service of a limited number of goals of vital importance*, indispensable for the survival of the individual and species (maintenance of homeostasis, fight and flight for survival, feeding, sexuality, progeny care, etc.), and the *acquired drives in service of an unlimited number of goals of not vital importance*, dispensable for the survival of the individual and species.

An acquired drive always originates in one way or another from an innate drive, which relation later becomes unrecognizable, thus, **nothing exists in the brain without a rational origin**. With the elaboration of the "glass cylinder seeking drive" (see inhn.org, Definitions), we hit upon the right method to study in rats the process of fixing an acquired drive. *In contrast to the*

rat, the mouse is unable to fix the glass cylinder seeking drive in the brain, which already suggests that the last step of crucial importance in the development of the mammalian brain was the appearance of the ability *to fix acquired drives.*

Vertebrates can be divided into three groups according to the mode of operation of their brain: (a) those which operate with innate drives only (the majority), (b) those with a limited ability to fix acquired drives (a minority); restricted manipulability of their behavior make them domesticable, and (c) the only one with unrestricted ability to fix acquired drives (*Homo sapiens sapiens*); thus, the manipulability of human behavior is infinite.

With the evolution of brains capable of acquiring drives, species appeared whose members could manipulate each other's behavior and act together. This was the condition *sine qua non* for the evolution of social living, a form of life that enabled the social group to qualitatively surpass the performance of the individual. It goes without saying that the training of skills to act in concert radically improved the quality of life. With the development of the human brain, a functional network with over 100 billion interrelated nerve cells and 10^{11} bit capacity arose. With this network, and with the inexhaustible capacity of the human brain to acquire drives, from the operation of which conscious perception is inseparable, life on earth reached the most sophisticated form of appearance. Furthermore, human beings, primarily social creatures, are building blocks in the creation of a gigantic product: **human society**, which represents the highest form of life on earth.

Since the human brain is born with the ability to envision a non-existing world but hasn't the foggiest idea how the world around him functions, creation of the still operating myths-directed human society could not be avoided. However, in order to survive, it was compelling to learn how the real world is functioning. To the end of the 18^{th} century, general knowledge progressed to a critical level. Since the age of enlightenment due to revolutionary changes in society (separation of Church and State), science and technology developed from strength to strength. Mankind strives after the final state: the rationally organized human society, grounded fully on scientific knowledge. Only a future global change in education, one based on the exact knowledge of the brain mechanisms responsible for the manipulation of human behavior, can finally lead to rationally directed society and terminate the still existing era where one hand destroys what the other hand has created.

Reply to "Deprenyl as an antidepressant, anti-Parkinson and anti-aging agent"

Reading your sober analysis regarding the therapeutic efficiency of a 10 mg daily dose of Selegiline/(-)-deprenyl (DEP) as an antidepressant and anti-Parkinson agent, interpretation is needed for why DEP, described in literature first in 1964, and now registered in more than 60 countries, marketed under more than 100 trade names, published in thousands of papers, still attracts attention. The recognition of the unique, complex pharmacological spectrum of this compound *in three consecutive phases*: the first in the 1960s, the second in the 1970s, and the third in the 1990s, each presenting a previously unknown and therapeutically useful effect, explains the peculiar development of the DEP-story.

DEP was synthetized in the early 1960s. The new compound first attracted attention as *the unique MAO inhibitor, free of the cheese effect*.

In the 1970s, DEP draw international attention as *the first selective inhibitor of B-type MAO*. The therapeutic daily dose of the drug is still 5-10 mg, the dose which blocks MAO-B in the brain.

In the 1990s, as summarized in my essay, the discovery of the catecholaminergic and serotoninergic *enhancer regulation in the mammalian brain*; the realization of β-phenylethylamine (PEA) and tryptamine as natural enhancer substances; the identification of DEP as the first PEA-derived synthetic enhancer substance; and the development of (2R)-1-(1-benzofuran-2-yl)-N-propylpentane-2-amine (BPAP), a tryptamine-derived synthetic enhancer substance, opened a promising new brain research domain.

A bi-modal, bell-shaped concentration effect curve is characteristic of the enhancer substances. As shown in Fig. 12, the optimum rat dose of DEP's 'specific' enhancer effect is 0.001 mg/kg and 0.25 mg/kg is the peak dose of the 'non-specific' enhancer effect. It deserves attention that the usually used 0.25 mg/kg DEP was selected to block completely MAO-B activity in the rat brain, and the therapeutic daily dose of DEP, 5-10 mg, was selected to block completely MAO-B in human brain. Thus, the human dose very likely has a second, overlooked effect: *it exerts the non-specific enhancer effect of DEP*. The share of the two simultaneously acting effects in the observed therapeutic benefits in humans treated daily with 5-10 mg DEP needs careful exploration.

Fig. 24 shows that BPAP, the most potent synthetic enhancer substance, exerts the specific enhancer effect in a dose as low as 0.0001 mg/kg, and 0.05 mg/kg is the peak dose of the non-specific enhancer effect.

The realization that during the uphill period of life, from weaning until sexual maturity, enhancer regulation in the catecholaminergic and serotonergic neurons works on a higher activity level, sexual hormones terminate the hyperactive phase, and this change is the beginning of the downhill period of life, seems to me to throw light on the essence of brain aging (Tables 3-7).

Maintenance on a low dose of DEP slows the decay of the brain engine's enhancer regulation. This safe preventive measure results in age-retarding, mental-energizing, performance-enhancing and longevity-promoting effects. Thus, it is not by chance that one finds on the internet a sea of enthusiastic reports on the benefits of taking daily 1 mg DEP as a prophylactic agent to slow brain aging. It is unfortunate that the implementation of a proper trial on healthy volunteers to measure exactly the anti-aging effect of DEP is still greatly needed.

The neurodegenerative diseases are incurable. We need to prevent or at least delay their manifestation. DEP is for sure primarily a catecholaminergic activity enhancer (CAE) substance. 1mg DEP daily slows the aging related decline of the catecholaminergic brain engine, thus improves the quality and prolongs the duration of life and may significantly delay the manifestation of neurodegenerative diseases.

Reply to "Theories that would be compatible with L'Deprenyl's anti-aging effect"

I followed your analysis with keen interest and thank you for your reasonable proposals for future research. Let me briefly summarize in light of our findings our present knowledge regarding the basic mechanism of the enhancer effect.

We selected the optimum doses of BPAP for the longevity study in shuttle box experiments using laboratory rats. The essence of the *in vivo* analysis was the measurement of the acquisition of a two-way conditioned avoidance reflex (CAR) in the shuttle box, and the inhibition of rats' learning ability with tetrabenazine-treatment (1 mg/kg sc.) which reversibly blocks the vesicular monoamine transporter 2 (VMAT2). Tetrabenazine depletes at least 90% of norepinephrine and

dopamine from their stores in the nerve terminals within 1 hour. The lower the degree of saturation in the transmitter pools the lower is the excitability of the neuron. Due to the weak performance of the catecholaminergic brain engine, activation of cortical neurons remains in tetrabenazine-treated rats below the level required for acquisition of a CAR. *However,* ***addition of 0.0001 mg/kg BPAP to 1 mg/kg tetrabenazine fully restored the learning ability of the rats*** (see 1.9 in essay, demonstrating that BPAP restored full activity of the catecholaminergic neurons in the presence of tetrabenazine.

It is worth mentioning in this context that DEP's protecting effect against a row of neurotoxic agents (6-OHDA, MPTP, DSP-4, AF64-A) is well known; and as shown in Fig. 12 DEP-treatment is also effective against tetrabenazine. Thus, the enhancer substances seem to transform the excitability of the enhancer sensitive neurons in a way that they are able to overpower various forms of neurotoxins.

It is common knowledge from electrophysiological studies with rodents and primates that the excitability and function of dopaminergic neurons are silent or spontaneously active. DEP or BPAP treatment keeps the catecholaminergic neurons on a higher activity level (Table 1-2 and Table 10-11). For example: *6.8 ± 0.18* nmol/g wet weight dopamine was released within 20 min from the substantia nigra isolated from saline treated rats and *14.8 ± 0.36* nmol/g wet weight dopamine was released within 20 min from the substantia nigra isolated from rats treated with a single dose of 0.0001 mg/kg BPAP. Similarly, a single dose treatment with 0.0005 mg/kg BPAP increased the release of norepinephrine from the isolated locus coeruleus within 20 min from *4.7 ± 0.10* (saline) to *15.4 ± 0.55* nmol/g wet weight; and a three-week treatment once daily with 0.0001 mg/kg BPAP acted similarly (the brain areas were isolated 24 hours after the last injection) (Table 10). *These results furnish evidence that the treatment of rats with 0.0001 mg/kg BPAP transformed the silent catecholaminergic neurons into spontaneous firing entities.*

Lifelong preventive medication obviously requires unique drug-safety. Due to their peculiar mechanism of action and safety margin, only the synthetic enhancer substances meet this requirement). BPAP exerts its specific enhancer effect in a subcutaneous dose as low as *0.0001 mg/kg*, and *20 mg/kg*, a 20.000-times higher dose is tolerated without any sign of toxic effect. This is truly an exceptional safety margin.

At present, DEP is the only synthetic enhancer drug in world-wide clinical use. In research and therapy, DEP celebrates a 50-year history. Very many patients are still treated permanently with the usual daily dose (10 mg) of DEP, as well as those who take 1 mg DEP daily to slow the aging-related decay of their catecholaminergic brain engine. They may slow, unaware, many unknown enhancer-sensitive mechanisms which pay a role in the improvement of the quality of life and longevity.

July 14, 2016

Joseph Knoll's reply (2) to Hector Warnes' comment

I fully agree with Dr. Hector Warnes' remarks (July 14, 2016) regarding the therapeutic efficiency of a 10 mg/day dose of selegiline. However, I wish to take this opportunity to point out the mistake that even deprenyl became well known in 1972 as the first selective inhibitor of B-type MAO and I discovered only 20 years later that the catecholaminergic activity enhancer (CAE) effect, exerted in low doses, is the primary important pharmacological effect of deprenyl; this is still not yet a matter of general knowledge.

Due to its specific pharmacological spectrum, deprenyl, the only synthetic β-phenylethylamine (PEA)-derivative devoid of the catecholamine-releasing property, enlightened me to the idea that the catecholaminergic neurons belong to the enhancer-sensitive brain regulations and led me to study this hitherto unknown life important mechanism in the mammalian brain.

Deprenyl is still registered in the text-books and used in research and therapy as the reference compound to block selectively MAO-B. Since the mid-1980s, however, deeper analysis of the characteristic enhancement of the catecholaminergic brain machinery in deprenyl-treated rats clarified that this effect is unrelated to the selective inhibition of MAO-B (Knoll, 1992). Thus, I started a structure-activity-relationship study to develop a deprenyl analog devoid of MAO inhibitory property and (-)-1-phenyl-2-propylaminopentane (PPAP), equally active on the catecholaminergic neurons as deprenyl, was selected for further studies (Knoll et al., 1992).

The first study, which demonstrated that multiple, low dose administration of deprenyl enhances catecholaminergic activity in the brain and this effect is unrelated to MAO-B inhibition, allowed for the discovery of the enhancer sensitive brain regulations (Knoll and Miklya, 1994).

PEA and its best known synthetic derivatives (amphetamine and methamphetamine) are strong releasers of catecholamines from their plasmatic pools. Even hordenine a PEA-class natural biogenic amine occurring in a number of plants, which like PEA, is binding to trace amine-associated receptors (TAAR1), is a releaser of catecholamines (Berry, 2007). Since the catecholamine releasing effect conceals the detectability of the enhancer-sensitive nature of the catecholaminergic neurons (Knoll, 2016). PEA's primary physiological function as a natural enhancer substance, as well as the fact that amphetamine and methamphetamine are, like deprenyl, PEA-derived synthetic enhancer substances, remained unknown. The later realization that tryptamine is, like PEA, a natural enhancer substance signaled the elaboration of BPAP, the most selective and potent synthetic enhancer substance currently known (Knoll et al.,1999).

Deprenyl is primarily a catecholaminergic enhancer (CAE) substance and is a weak enhancer of serotonergic neurons. BPAP, as a tryptamine-derivative, is a highly potent enhancer of serotonergic neurons, but it is, even as a CAE substance, much more potent than deprenyl. The catecholaminergic and serotonergic neurons were studied as the first models of the enhancer-sensitive brain regulations (Knoll, 2005).

With regard to the molecular mechanism of action of the enhancer-substances, we found later that BPAP, injected in a dose of 0.0001 mg/kg, reversed the decrease in the electrical stimulation-induced [^{3}H]dopamine release, evoked by 1 mg/kg of tetrabenazine in superfused rat striatal slices. Tetrabenazine is a VMAT2 inhibitor proposed to interact with the extravesicularly located dihydrotetrabenazine binding site that is distinct from the dopamine uptake site on VMAT2. Moreover, tetrabenazine also binds to intra-vesicular dopamine release sites of VMAT2, exhibiting high and low sensitivity in binding affinity. Both the extra- and intra-vesicular VMAT2 dopamine uptake and release sites may be involved in BPAP's effect. Furthermore, BPAP, acting as a substrate inhibitor of VMAT2, may compete with dopamine for uptake into the vesicle, and may exhibit a low affinity binding to the dopamine uptake site on VMAT2, as suggested by its poor activity on resting [^{3}H]dopamine release in superfused striatal slices of the rat. The biphasic concentration-response curve for BPAP to release [^{3}H]dopamine following electrical stimulation

that fits a two-site model of interaction supports the interaction of two different intra-vesicular sites: a high affinity (picomolar) site and a low affinity (μmolar) dopamine release site. A binding to the high affinity dopamine release site represents the specific catecholaminergic activity enhancer activity of BPAP (Knoll et al., 1999), whereas the low affinity site is responsible for BPAP's non-specific enhancer effect of on [^{3}H]dopamine release. Taking into account the dopamine uptake and release sites on VMAT2, to which tetrabenazine and BPAP bind, we concluded that the observed interaction of these two drugs in [^{3}H]dopamine release may be related to a binding of BPAP to the high affinity intra-vesicular dopamine release site, which is, on the other hand, also sensitive to the tetrabenazine, the vesicular inhibitor. All in all, the molecular mechanism of the enhancer effect clarifies BPAP's highly characteristic bi-modal, bell-shaped concentration effect curves (Knoll et al., 2002) and testifies to the idea that the enhancer-sensitive brain regulations represent a promising new brain research domain.

The discovery of the enhancer regulation in the mammalian brain, the study of the catecholaminergic and serotonergic neurons as enhancer sensitive brain regulations, the identification of PEA and tryptamine as natural enhancer substances, the proof that selegiline/(-)-deprenyl (DEP) is the first PEA-derived synthetic enhancer substance devoid of the catecholamine releasing property, and the development of (2R)-1-(1-benzofuran-2-yl)-N-propylpentane-2-amine (BPAP) allowed for promising new brain-research (Knoll 2001, 2003, 2012; Miklya, 2016).

Prior to the discovery of the catecholaminergic activity enhancer effect of DEP and the discovery of the enhancer regulation in the mammalian brain, the hypothesis was proposed in 1981 that a progressively developing catecholaminergic and trace-aminergic deficiency is responsible for the biochemical lesion in the aging brain which leads to the age-related decline in sexual and learning performance and ultimately natural death soon proved that this effect of DEP is unrelated to the inhibition of MAO-B (Knoll, 1982; Knoll and Miklya, 1995).

Enhancer substances keep the catecholaminergic neurons on a higher activity level. For example: 6.8±0.18 nmol/g wet weight dopamine was released within 20 minutes from the *substantia nigra* isolated from saline treated rats and 14.8±0.36 nmol/g wet weight dopamine was released within 20 minutes from the *substantia nigra* isolated from rats treated with a single dose of 0.0001 mg/kg BPAP. Similarly, a single dose treatment with 0.0005 mg/kg BPAP increased the release of norepinephrine from the isolated *locus coeruleus* within 20 minutes from 4.7±0.10

(saline) to 15.4±0.55 nmol/g wet weight; and a three-week treatment once daily with 0.0001 mg/kg BPAP acted similarly (the brain areas were isolated 24 hours after the last injection). These *ex vivo* results from studies using isolated discrete rat brain regions are in complete harmony with the results of the *in vivo* shuttle box experiments and furnish unequivocal evidence that the *treatment of rats with 0.0001 mg/kg BPAP transformed the silent catecholaminergic neurons into spontaneous firing entities.*

It is worth remembering the origin of the enhancer regulation concept. "An eagle pounces upon the chosen victim with lightning speed. Reacting accordingly is a life-and-death matter. Both the attacker and the victim have a split second to respond. This promptness of activation in assault/escape behavior inspired the working hypothesis in the mid-1980s that an unknown, life important, enhancer regulation, capable to momentarily increase neuronal excitability, might operate in the mammalian brain. Since the cerebral catecholaminergic machinery is responsible for the general activation of the cortex, it was reasonable to expect that the catecholaminergic brain engine must be endowed with this capacity" (Knoll, 2016).

It is well known from studies with rodents and primates that dopaminergic neurons are silent or spontaneously active (Marinelli, 2006). Our finding that the treatment of rats with 0.0001 mg/kg BPAP transformed the silent catecholaminergic neurons in the enhancer-sensitive dopaminergic neurons into spontaneous firing entities explains the promptness of activation in assault/escape behavior. It is hard to overestimate the therapeutic consequences of the fact that influenced by 0.0001 mg/kg BPAP, this highly potent synthetic enhancer substance was capable of dramatically transforming dopaminergic neurons's operation.

One of the most crucially important conclusions regarding the unknown life important physiological effects of the catecholaminergic and serotonergic neurons is that these enhancer-sensitive regulations work in the uphill period of life, from weaning until sexual maturity, on a significantly higher activity level. Sexual hormones (estrone, testosterone) return the enhancer regulation to the pre-weaning level, putting into action the downhill period of life and the aging-related slow decay of the enhancer regulation continues until death (Knoll and Miklya 1995; Knoll et al., 2000). Thus, maintenance during the downhill period of life on a proper low dose of a synthetic enhancer substance slows the aging related decay of the enhancer sensitive brain regulations, improves the quality of life in the latter decades, prolongs life and delays/prevents the

manifestation of enhancer-regulation-dependent illnesses, signaling that the enhancer regulation, due to aging-related decay, already surpassed the critical threshold (Knoll, 1994). For example, we lose 13% of our dopamine in the decade after age 45. In the healthy population, the calculated loss of striatal dopamine is about 40% at the age of 75 which is about the average lifetime. As symptoms become visible only after the unnoticed loss of about 70% of striatal dopamine, in diagnosing Parkinson's disease the neurologist selects subjects with the most rapidly aging striatal dopaminergic system (about 0.1% of the population.)

At present DEP, the PEA-derived CAE substance is the only safe synthetic enhancer drug in world-wide clinical use. In research and therapy, DEP celebrates a 50-year history. Masses of patients are still treated with a daily dose (10 mg) of DEP, and masses of people take 1 mg DEP daily to slow the aging-related decay of their catecholaminergic brain engine (Miklya, 2016).

Decades ago, based on the concept that the long term administration of DEP may improve the quality of life in the declining years (Knoll, 1982) and this effect of DEP is unrelated to the inhibition of MAO-B (Knoll and Miklya, 1995), a retrospective analysis in parkinsonian patients was performed. The long term (nine years) effect of treatment with Madopar alone (N=177) or in combination with DEP (N= 564) revealed a significant increase in life expectancy in Madopar+DEP group regardless of the significant demographic differences between the two groups (Birkmayer et al., 1985).

Lifelong preventive medication obviously requires unique drug-safeness. Due to their peculiar mechanism of action and safety margin, only the synthetic enhancer substances adhere to this requirement. BPAP exerts its specific enhancer effect in a subcutaneous dose as low as 0.0001 mg/kg, and 20 mg/kg, a 20 times higher dose, is tolerated without any sign of toxic effect. This is truly an exceptional safety margin (Knoll and Miklya, 1994).

As shown in a recent longevity study performed with low doses of deprenyl (Knoll and Miklya, 2016), the regularly used 10 mg/day human dose has two effects: it inhibits MAO-B and exerts its non-specific enhancer effect. It remains for the future to clarify, in retrospect, the participation of MAO-inhibition versus enhancer effect in the benefits observed in patients treated with deprenyl.

To illustrate with a final example the consequences of the discovery of the enhancer-sensitive brain regulations and the development of the first synthetic enhancer-substances I am citing a recently published example demonstrating the anti-aging effect of BPAP. We treated rats daily with saline versus 0.0001 mg/kg BPAP and measured in the shuttle box their ability to fix a conditioned avoidance reflex (CAR). Due to aging of the dopaminergic neurons, learning ability is subject to an age-related decline. We found in our recent study that the three month old group of saline-treated rats worked with full capacity and built on the fifth day of training, on average, nearly 90% of the CARs. Due to aging of the dopaminergic neurons, the group of 18-month-old saline-treated rats reached on the fifth day of training, on average, only less than 30% of the CARs. *However, the group of 18-month-old rats treated daily with 0.0001 mg/kg BPAP produced on the fifth day of training, on average, more than 90% of the CARs* (Knoll and Miklya, 2016). As discussed above, 0.0001 mg/kg BPAP specifically stimulates the enhancer-sensitive dopaminergic neurons and we surprisingly found that by treating rats daily with a proper synthetic enhancer substance, 18-month-old rats remained as efficient learners in the shuttle box as the most active three-month-old rats. This is an unprecedented novelty.

As published step by step since the 1950s and summarized in monographs (Knoll 1969, 2005, 2012, 2016), the essence of our findings leading finally to the discovery of the enhancer-sensitive brain regulations and the development of deprenyl and BPAP, open a hitherto unknown possibility to improve the quality and the duration of human life via slowing brain aging.

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February 16, 2017

Hector Warnes' response to Joseph Knoll's replies

I am most grateful for Professor Knoll's replies to my comments! He obviously has the profile of a great scientist and a life long researcher into enhancer regulation in the mammalian brain. Deprenyl was, the first selective inhibitor of B-type MAO without the cheese effect. Over several decades Professor Knoll's attention was drawn to its catecholaminergic activity enhancer (CAE) effect.. It led him further to develop other synthetic enhancer substances such a the beta-phenylethylamine (PEA) and later the BPAP, a tryptamine-derived synthetic enhancer substance:

"The realization that during the uphill period of life, from weaning until sexual maturity, enhancer regulation in the catecholaminergic and serotoninergic neurons work on a higher activity level, sexual hormones terminate the hyperactive phase, and this change is beginning a downhill period of life seems to me to throw light on the essence of human aging." I would add that there are several changes that take place in the organism during the inexorable process of aging besides the ones that Professor Knoll name as 'essential': DNA damage, oxidative stress, proteotoxic stress, telomere shortening, increase of free radicals that may damage cells particularly its mitochondria, impairment of the immune response, susceptibility to cancer, vascular disease and others pathologies which Professor Knoll may not considered them as 'essentials' but rather secondary to the CAE.

Professor Knoll has shown in 1992 that enhancer substances (CAE) given to rats in lifelong maintenance doses significantly prolongs their life. He went further by depleting (over 90%) of the rats norepinephrine and dopamine from their stores in nerve terminals by using tetrabenazine. The depletion was reversed by the treatment of rats with BPAP.

As far as I am aware no clinical studies were done in humans that confirms his findings in rats. There has been abundance of new Senotherapeutic agents that have raised hope about improving the quality and the life span of humans but later were dismissed or shown to require further investigations. I would like just to mention those anti-aging agents that are flooding the market such as antioxidants (free radicals which may damage the cells), growth hormones, L-carnitine, telomerase (which mantains the telomere length), melatonin, carnosine, metformin,delta sleep inducing peptide (as we know delta sleep decreases to a minimal percentage with aging), caloric restriction mimetic drugs (rapamycin), mitoq (a mitochondria targeted antioxidant), metformin and even stem cells. Many were discarded such as Aslan's treatment with procaine or more recently resveratrol.

I would admit my own ignorance regarding the lifelong meticulous research of Professor Knoll. I would like to ask him if he was able to compare the same strain of rats treated chronically with CAE with another number of rats of the same strain treated with another anti-aging compound that has been most promisory. I would not mind myself taking Deprenyl to ameliorate the usual symptoms of old age not related to illness.

August 24, 2017

Joseph Knoll's response 1 to Hector Warnes' response 1

In response to your question whether we compared the antiaging effect of deprenyl with antiaging effect of other antiaging drugs in the same strain rats, we compared the pharmacological profile of anti-aging compounds with deprenyl (DEP) and also measured their CAE effect.

An example:

From therapeutic perspective, the value of treating PD with DEP was a significant finding in the USA, first published by Tetrud and Langston in 1989. They concluded that DEP-treatment delayed the need for levodopa therapy. In their study the average time until levodopa was needed was 312.1 days in the placebo group and 548.9 days for patients in the DEP group. This finding was already confirmed in the 1989 DATATOP multicenter study by the Parkinson Study Group. Important multicenter studies, such as the French Selegiline Multicenter Trial (FSMT), the Finnish Study the Swedish Parkinson Study Group and the Norwegian-Danish Study Group, confirmed the usefulness of the drug in *de novo* PD.

When the DATATOP study was planned, DEP's enhancer effect was unknown, so the organizers' hypothesis was that the activity of MAO and the formation of free radicals predispose patients to nigral degeneration and contribute to the emergence and progression of PD. In accord with their working hypothesis, they expected that DEP, the MAO inhibitor, α-tocopherol, the antioxidant, and the combination of the two compounds would slow the disease's clinical progression.

They selected patients with early, untreated PD and measured the delay in the onset of disability necessitating levodopa therapy. In the first phase of the trial, 401 subjects were assigned to □-tocopherol or placebo and 399 subjects were assigned to DEP, alone or with □-tocopherol. *Only 97 subjects who received DEP reached the "end" of the trial* (i.e., the onset of disability necessitating levodopa therapy) *during an average 12 months of follow-up compared with 176 subjects who did not receive DEP. The risk of reaching the end of the trial was reduced by 57% for the subjects who received DEP, and these patients also had a significant reduction in their risk of having to give up full-time employment.* Following the course of changes, the authors concluded in their next paper that DEP, but not □-tocopherol, delayed the onset of disability associated with early, otherwise untreated PD.

The unexpected outcome of the DATATOP study, the finding that DEP delayed the need for levodopa therapy, but □-tocopherol fell short of expectation, clearly proved that DEP exerts an unknown pharmacological effect of basic importance and □-tocopherol is devoid of this effect. Now we know that DEP as a CAE substance is an enhancer of the impulse propagation mediated release of catecholamines. *A comparative pharmacological analysis of DEP and □-tocopherol proved that □-tocopherol is devoid of the enhancer effect* (Miklya, Knoll B, Knoll J 2003).

This conclusion was also supported by the clinical trial with rasagiline, the selective MAO-B inhibitor, performed by the Parkinson Study Group. The trial revealed that unlike the early selegiline trials, rasagiline failed to demonstrate a decreased need for levodopa. *Similar to □-tocopherol, rasagiline is also devoid of the CAE effect of DEP* (Miklya 2014).

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October 19, 2017

Hector Warnes' response 2 to Joseph Knoll's response 1

I am grateful to Professor Knoll for his partial response to my question. He cited two Datatop Multicenter studies of early Parkinson disease which demonstrated that L-Deprenyl, Selegiline, a MAO-B inhibitor, delayed the need for Levo-Dopa therapy, retards the spread of the disease and the onset of disability, while the anti-oxidant tocopherol (vitamin E) and/or rasagiline had no effect.

Parkinson's Disease, a neurodegenerative disease, appears usually after the age of 60 and is attributed to a loss of dopaminergic neurons within the substantia nigra. It has been shown that most changes in Parkinson's Disease can be also found in the aging population, such as genetic factors; increased oxidative and inflammatory damage; dysfunction of intracellular calcium metabolism; and in protein degradation and mitochondrial activity. The term "brain cellular senescence" has been used. It appears that the most outstanding difference is in the gene expression of alpha-synuclein (Park 1) and the deposit of iron in the substantia nigra. The particular pigmentation of the substantia nigra is due to neuromelatin's accumulation. Neuromelatin binds iron and chelates it. In the general population, about 1% of people over 60 suffer from Parkinson's Disease while more than 5% of them over 80 develop it. An excellent review of the relationship

between ageing and Parkinson's Disease was published in 2014 by A. Reeve, E. Simcox and D. Turnbull.

As a clinical psychiatrist, I do welcome new antidepressants and, from experience, have tested L-Deprenyl in Transdermal patches, which has been shown to be effective in a group of depressive patients. We have not as yet identified the specificity of the various antidepressants with different locus of activity in the brain circuits and still we have to deal with a great number of chronic depressives who are refractory to several antidepressants, even to a combination of them. (I am sure Professor Knoll would sort out the characteristics of the group of patients who are responsive to MAO-B inhibitors.) It was also found that L-Deprenyl has neuroprotective action and improves cognitive functions, but so far it has not been clearly shown that it increases life span. L-Deprenyl metabolizes dopamine and phenylethylamine which are considered enhancer substances. As a clinician, I would worry about the side effects in the elderly population, particularly induced by drug interactions. It should not be given with narcotics, with oral oestrogens, with SSRIs inhibitors and so on.

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Reeve A, Simcox E, Turnbull D. Ageing and Parkinson's disease: why is advancing age the biggest risk factor? Ageing Research Reviews 2014; 14: 19-30.

January 4, 2018

Joseph Knoll's response 2 to Hector Warnes' response 2

Clinicians are still convinced that the selective inhibition of MAO-B is primarily responsible for the beneficial therapeutic effects of selegiline/(-)-deprenyl (DEP). This misunderstanding is due to the negligence of the gradual recognition of the complicated pharmacological spectrum of DEP.

The first phase in selegiline (DEP) history was the recognition that DEP is free of the 'cheese effect' (Knoll et al. 1968).

This was finally proved in 1988. We compared all the known MAO inhibitors' response to tyramine on rabbit arterial strips. Not only the best known and regularly used MAO inhibitors, but also all the newly published, and at that time, less known compounds were studied. ***Only DEP inhibited the response to tyramine*** (Abdorubo et al. 1988)**.**

The second phase was the recognition that DEP is the first selective inhibitor of MAO-B (Knoll and Magyar 1972).

Prior to the discovery of the catecholaminergic activity enhancer (CAE) effect of DEP (Knoll 1998), *it was my firm belief that the selective inhibition of MAO-B is responsible for the drug's beneficial therapeutic effects.* However, further studies, which for the first time revealed the true aphrodisiac effect of 0.25 mg/kg DEP, led to an unexpected observation which raised doubts regarding the supposed leading role of the selective inhibition of MAO-B in DEP's therapeutic effects (Knoll 1982).

At that time when we discovered DEP's true aphrodisiac effect, we developed U-1424, a new indane-derived potent selective inhibitor of MAO-B (Knoll et al. 1978). We performed with U-1424 exactly the same experiment as with DEP on sluggish, aged rats and *found that the new compound did not possess an aphrodisiac effect* (Knoll 1982). *I concluded in my lecture that DEP exerts its aphrodisiac effect by more than one mechanism* (Knoll 1982). For me, this finding was a serious warning that we needed to clarify DEP's unknown mechanism, obviously unrelated to MAO-B inhibition.

We developed (-)-1-phenyl-2-propylaminopentane (PPAP), the DEP-analog containing a propyl-group attached to the nitrogen instead of the propargyl-group. The propyl-group is unable to covalently bind with the flavin in MAO-B rather than the propargyl-group in DEP. Thus, PPAP leaves MAO-B activity unchanged, however, as a central stimulant of the catecholaminergic neurons, PPAP proved to be as potent as DEP (Knoll et al. 1992).

The third phase was the recognition that DEP is a PEA-derived synthetic enhancer substance (Knoll et al. 1996).

Discovery of the enhancer-sensitive regulations in the mammalian brain opened a new domain in brain research (Knoll 2003, Knoll 2005). An enhancer-sensitive neuron is defined as

one capable to change excitability and work immediately on a significantly higher activity level in the presence of a natural or synthetic enhancer substance (Knoll 2005).

The catecholaminergic and serotonergic neurons and their natural enhancers, β-phenylethylamine (PEA) for the catecholaminergic neurons and tryptamine for the serotonergic neurons, were identified as enhancer-sensitive brain regulators and selected as the first models to study the characteristics of the enhancer-sensitive brain regulations. Two synthetic enhancers were developed for the pharmacological analysis: Selegiline/(-)-deprenyl (DEP), the PEA-derived synthetic catecholaminergic activity enhancer (CAE) substance (Knoll 1998) and (2R)-1-(1-benzofuran-2-yl)-N-propylpentane-2-amine (BPAP) the tryptamine-derived synthetic enhancer substance (Knoll et al. 1999).

BPAP, the selective and much more potent synthetic enhancer than DEP, is preferentially used as a specific marker to detect unknown enhancer-sensitive brain regulations. A bi-modal, bell-shaped concentration effect curve is characteristic to the enhancer substances. This peculiar behavior brought the distinction of the *"specific"* and *"non-specific"* enhancer effect to perfection. The bi-modal, bell-shaped nature of the enhancer effect, confirmed on the cultured rat hippocampal neurons (Knoll et al. 1999), was first precisely analyzed on the isolated locus coeruleus of rats (Knoll et al. 2002). In this test, BPAP enhanced the activity of the noradrenergic neurons in the femto/picomolar concentration range with a peak at 10^{-13} M ("specific" enhancer effect), and also in a 10 million times higher concentration range with a peak at 10^{-6} M ("non-specific" enhancer effect).

Interaction with distinct sites on vesicular monoamine transporter-2 (VMAT2) is the main mechanism of action of the enhancer substances which clarifies the highly characteristic bi-modal, bell-shaped concentration effect curves of DEP and BPAP (Knoll et al. 2017).

The main effect of DEP is the enhancer effect

It is unquestionable that since the early 1960s DEP's story would be full of surprises. DEP-research catalyzed us to the discovery of the enhancer regulation in the mammalian brain, to the realization that the catecholaminergic and serotonergic neurons are enhancer-sensitive units, and to the development of BPAP.

A recent study presents evidence that the enhancer effect of DEP and BPAP are responsible for the prolongation of mammalian life (Knoll and Miklya 2016). Rats treated three times a week with 0.0001 mg/kg BPAP, which is the peak dose exerting its "*specific" enhancer effect*, significantly prolonged the rat life. This study also showed that the 0.25 mg/kg dose of DEP, used from the beginning in the longevity studies, has two effects: *it is the peak dose which completely blocks MAO-B in the brain, and it is also the peak dose which exerts the non-specific enhancer effect of DEP* (Knoll and Miklya 2016).

Since the presently used 10 mg daily dose of DEP in therapy was originally selected as the one equivalent with the dose used in animals, it remains for the future to clarify the role of the non-specific enhancer effect of DEP in the therapeutic benefits observed in the last decades.

That the main effect of DEP is the enhancer effect is unquestionable.

As already noted, 0.25 mg/kg DEP is the peak concentration that elicits in rats both MAO-B inhibition and the "*non-specific"* enhancer effect. We established at the very beginning of the planned clinical trials with DEP that the 0.25 mg/kg dose of DEP, which selectively and completely blocks MAO-B activity in the rat brain, is equivalent with 10 mg/day DEP in humans, and this remains the standard daily therapeutic dose of DEP. Considering DEP's already exactly verified pharmacological profile, it is obvious that DEP exerts the same two effects in humans as in rats.

As a matter of fact, it was the Deprenyl And Tocopherol Antioxidant Therapy Of Parkinsonism/Parkinson Study Group (PSG) which published the DATATOP study's results that DEP has a beneficial influence on the natural history of PD, which clearly proved, in light of the discovery of the CAE effect, that only the enhancer effect of DEP can be responsible for this unexpected, unknown and unique benefit. Tetrud and Langston (1989) published first in *Science* that DEP-treatment delayed the need for levodopa therapy. They found that the average time until levodopa was needed was **312.1** days in the placebo group and **548.9** days for patients in the DEP group. This finding was immediately confirmed (Parkinson Study Group 1989).

The original 1989 Science paper that reported the finding that DEP treatment is changing the natural history of PD was soon further confirmed by important multicenter studies, such as the French Selegiline Multicenter Trial (FSMT) (Allain et al. 1991), the Finnish Study (Myttyla et al.

1992), the Swedish PSG (Palhagen et al. 1998), and the Norwegian-Danish Study Group (Larsen et al. 1999).

When the DATATOP study was planned, DEP's enhancer effect was unknown, so the organizers hypothesis was that the activity of MAO and the formation of free radicals predispose patients to nigral degeneration and contribute to the emergence and progression of PD. In accordance with their working hypothesis, they expected that DEP, the MAO inhibitor, α-tocopherol, the antioxidant, and the combination of the two compounds would slow the disease's clinical progression.

They selected patients with early, untreated PD and measured the delay in the onset of disability necessitating levodopa therapy. In the first phase of the trial, 401 subjects were assigned to α-tocopherol or placebo and 399 subjects were assigned to DEP, alone or with α-tocopherol. Only 97 subjects who received DEP reached the "end" of the trial (i.e., the onset of disability necessitating levodopa therapy) during an average 12 months of follow-up compared with 176 subjects who did not receive DEP. The risk of reaching the end of the trial was reduced by 57% for patients who received DEP, and these patients also had a significant reduction in their risk of having to give up full-time employment (Parkinson Study Group 1989). Following the course of changes, the authors concluded in their next paper that DEP, *but not α-tocopherol*, delayed the onset of disability associated with early, otherwise untreated PD (Parkinson Study Group 1993). But over time, the DATATOP study also revealed that DEP did not reduce the occurrence of subsequent levodopa-associated adverse effects in patients. This fact still needs serious consideration (Parkinson Study Group 1996).

Idiosyncratic prescribing of DEP in combination with levodopa already led to false conclusions (Knoll 2010). Due to the inhibition of MAO-B, DEP-treatment allows for a 20-50% decrease in levodopa dose needed in PD. In patients who need levodopa, however, there is always a risk that the administration of DEP will enhance the side effects of levodopa which can only be avoided by properly decreasing the levodopa dose according to individual sensitivity.

An example of a multicenter clinical trial with improper combination of levodopa which led to confusion and misinterpretation, was the one performed by the PD Research Group in the United Kingdom (PDRG-UK) (Lees 1995). Quite unexpectedly, this group published an alarming paper claiming that parkinsonian patients treated with levodopa combined with DEP show an

increased mortality in comparison with the patients treated with levodopa alone. This finding was in striking contradiction to all other studies published in a variety of countries. Comments uniformly pointed to substantial overdosing of levodopa (Dobbs et al. 1996, Knoll 1996, Olanow et al. 1996).

The outcome of the DATATOP study, the finding that DEP delayed the need for levodopa therapy, but α-tocopherol fell short of expectation, clearly proved that DEP exerts an unknown pharmacological effect of basic importance and α-tocopherol is devoid of this effect. Now we know that DEP, as a CAE substance, is an enhancer of the impulse propagation mediated release of catecholamines. A comparative pharmacological analysis of DEP and α-tocopherol proved that α-tocopherol is devoid of the enhancer effect (Miklya et al 2003). Since 0.25 mg/kg DEP selectively blocks MAO in the brain and also exerts in the same dose the non-specific enhancer effect (Knoll and Miklya 2016), it is obvious that DEP's CAE effect was responsible for the delayed levodopa need (Knoll 2010).

This conclusion was also supported by the clinical trial with rasagiline, performed by the PSG. The trial revealed that unlike the early selegiline trials, rasagiline failed to demonstrate a decreased need for levodopa (Parkinson Study Group 2002). Even the results of additional studies (Olanow and Rascol 2010, Ahlskog and Uitti 2010), led to the conclusion that "based on current evidence, rasagiline cannot be said to definitely have a disease-modifying effect" (Robottom 2011). Similar to α-tocopherol, neither lazabemide nor rasagiline, the two selective MAO-B inhibitors used in PD, are also devoid of the CAE effect of DEP (Miklya 2014).

Since the mid-1980s, further analysis of the characteristic enhancement of the catecholaminergic brain machinery in DEP-treated rats rendered probable that this effect is unrelated to the selective inhibition of MAO-B. The development of PPAP, the DEP-analog devoid of a MAO inhibitory property, and an equally active stimulant of the catecholaminergic neurons as DEP, verified this suggestion (Knoll 1992). The first study which demonstrated that multiple, low dose administration of DEP enhances catecholaminergic activity in the brain and this effect is unrelated to MAO-B inhibition allowed for the discovery of the enhancer sensitive brain regulations (Knoll and Miklya 1994). PEA and its best-known synthetic derivatives (AM and MAM) are strong releasers of catecholamines from their plasmatic pools. Since the catecholamine releasing effect conceals the detectability of the enhancer-sensitive nature of the catecholaminergic

neurons (Knoll 2016), DEP's primary physiological function as a natural enhancer substance, as well as the fact that AM and MAM are, like DEP, PEA-derived synthetic enhancer substances, remained unknown.

The later realization that tryptamine is like PEA, a natural enhancer (Knoll 1994), signaled the elaboration of BPAP as the most selective and potent synthetic enhancer substance currently known (Knoll et al. 1999).

The discovery of the enhancer-sensitive brain regulations and the development of synthetic enhancer substances clarified that both the developmental and post-developmental phases of mammalian life are under strict control of the enhancer-sensitive brain regulations. During the developmental period of life, from weaning until sexual maturity, the enhancer-sensitive neurons work on significantly higher activity level (Knoll and Miklya 1995). Sexual hormones immediately restore the pre-weaning low level of the enhancer-sensitive brain regulations and activate the post-developmental (aging) phase; due to the slow unbroken loss of the natural enhancers, the regressive effects of brain aging continue until death (Knoll et al. 2000).

The preliminary observations that enhancer-sensitive neurons do not age suggested that synthetic enhancer substances might prevent the regressive effects of brain aging. A carefully performed longevity study verified the suggestion (Knoll and Miklya 2016).

Since the enhancer-sensitive dopaminergic neurons are primarily responsible for the rat's learning ability and we also know that, similar to the human brain, the dopaminergic neurons belong to a very rapidly aging brain-system (Knoll 2010), we selected the learning test to prove that the dopaminergic neurons do not age. We treated subcutaneously, three times weekly, groups of rats from sexual maturity until death with saline versus 0.0001 mg/kg BPAP, the peak dose of the synthetic enhancer with the specific enhancer effect. We measured in the shuttle box rats' ability to fix during a 5-day daily training a conditioned avoidance reflex (CAR). We found in this study that 3-month-old saline-treated rats worked with full capacity in the shuttle box and built on the 5th day of training an average of nearly 90% of the possible 100% of CARs. Due to aging of the dopaminergic neurons, the 18-month-old saline-treated rats reached on the 5th day of training an average of less than 30% of the possible 100% of CARs. **However, the group of 18-month-old rats treated from sexual maturity until death three-times weekly with 0.0001 mg/kg BPAP reached on the 5th day of training an average of over 90% of the possible 100% of**

CARs (Knoll and Miklya 2016). The proof that BPAP-treatment fully prevented the aging-related decay of the dopaminergic neurons shows promise that we may safely counteract in the future the regressive effects of brain aging and, thus, improve the quality and prolong the duration of human life.

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January 25, 2018

Hector Warnes' response 3 to Joseph Knoll's response 2

Professor Knoll has been passionately devoted over fifty years to his research on Deprenyl and several of its synthetic derivatives. For the last one, BPAP, after meticulous research on rats, Professor Knoll stated that it significantly prolonged the rat's life. BPAP is a Deprenyl analog which acts by centrally stimulating the catecholaminergic neurons, therefore acting like a synthetic enhancer substance.

The more I read Professor Knoll the more I understand his life-long search for enhancers which boost dopamine levels. Dopamine is a neurotransmitter which is involved in movement,

coordination, sex drive, pleasure and cognition. It also has neuroprotective properties and increases the level of endogenous antioxidants.

Deprenyl, by blocking the MAO-B, prevents the breakdown of dopamine and phenylethylamine. A disruption of neurotransmitter synthesis is central in many pathologies including Parkinson's disease, depression, neurodegenerative disorders and so on.

I am aware of several research lines on the telomere which are the "caps" of the chromosomes and are vital for the cell integrity and length stabilization. Its shortening is a signal of progressive ageing or can also point to several pathological states. An enzyme, the telomerase helps to preserve the telomere length. In 2008 N. V. Osipov published an abstract of an original research paper published in Russian. Using Wistar rats and "a newly developed pharmacological method" (not named), Osipov demonstrated with that while rats from the control group died at the age of 1 year 7 months - 1 year 8 months, rats from the experimental group died at the age of 2 years 4 months. Osipov also used the Morris's labyrinth water test and measured the telomere's length stabilization. Perhaps Professor Knoll knows if Osipov used an analog of Deprenyl in his research.

There have been other substances tested for their potential to prolong the span of life. They include melatonin which improves sleep, is anti-stress and upregulates the immune system; fullerene, an extract of olive oil containing phenotic antioxidants; myricetin, a natural flavonol; and ghrelin which was shown to prolong life in the mouse. The latter is secreted by the stomach in response to fasting. Low caloric diet has also been shown to prolong life.

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April 26, 2018

Joseph Knoll's additional response 2 to Hector Warnes' response 2

You refer in your comment to the DATATOP study papers of the Parkinson Study Group, published in 1989. In agreement with the still generally accepted view, you refer to the

(-)-deprenyl/selegiline (DEP)-treatment induced delayed need for levodopa-therapy as a consequence of DEP-induced MAO-B inhibition.

We presented in a recent longevity study experimental evidence that rats treated daily with 0.0001 mg/kg (2*R*)-1-(1-benzofuran-2-yl)-*N*-propylpentane-2-amine (BPAP), the peak dose which exerts the specific enhancer effect, lived significantly longer than their saline-treated peers and BPAP was more potent than DEP in extending the lifespan of rats (Knoll and Miklya 2016). This was the proof that the enhancer effect is responsible for BPAP-induced life extension.

When the DATATOP study was planned in the late 1980s, DEP's enhancer effect was unknown. The organizer's hypothesis for the DATATOP study was the concept that the activity of MAO and the formation of free radicals predispose patients to nigral degeneration and contribute to the emergence and progression of Parkinson's disease (PD). In accordance with their working hypothesis, they expected that DEP (the MAO inhibitor), α-tocopherol (the antioxidant) and the combination of the two compounds would slow the disease's clinical progression.

They selected patients with early, untreated PD and measured the delay in the onset of disability necessitating levodopa therapy. In the first phase of the trial, 401 subjects were assigned to α-tocopherol or placebo and 399 subjects were assigned to DEP, alone or with α-tocopherol. Only 97 subjects who received DEP reached the "end" of the trial (i.e., the onset of disability necessitating levodopa therapy) during an average 12 months of follow-up compared with 176 subjects who did not receive DEP. The risk of reaching the end of the trial was reduced by 57% for patients who received DEP and these patients also had a significant reduction in their risk of having to give up full-time employment (Parkinson Study Group 1989). Following the course of changes, the authors concluded in their next paper that DEP, ***but not α-tocopherol***, delayed the onset of disability associated with early, otherwise untreated PD (Parkinson Study Group 1993). Over time, however, the DATATOP study also revealed that DEP did not reduce the occurrence of subsequent levodopa-associated adverse effects in patients. This fact still deserves serious consideration (Parkinson Study Group. 1996).

The outcome of the DATATOP study, the finding that DEP delayed the need for levodopa therapy, but α-tocopherol fell short of expectation, clearly proved for us that DEP exerts an unknown pharmacological effect of basic importance and α-tocopherol is devoid of this effect.

We soon realized that DEP is a PEA-derived catecholaminergic activity enhancer (CAE) substance, an enhancer of the impulse propagation mediated release of catecholamines. A comparative pharmacological analysis of DEP and α-tocopherol proved that α-tocopherol is devoid of the enhancer effect (Miklya et al. 2003). At 0.25 mg/kg DEP selectively blocks MAO-B in the brain and also exerts, in the same dose, the non-specific enhancer effect (Knoll and Miklya 2016). Furthermore, since DEP prolongs the lifespan of rats in 0.001 mg/kg dose, it is obvious that the enhancer effect of DEP is responsible for the observed delay of levodopa need (Knoll 2012).

This conclusion was also supported by the clinical trial with rasagiline performed by the Parkinson Study Group. The trial revealed that unlike the early selegiline trials, rasagiline failed to demonstrate a decreased need for levodopa (Parkinson Study Group 2002). Even the results of additional studies (Ahlskog and Uitti 2010; Olanow and Rascol 2010) led to the conclusion that "based on current evidence, rasagiline cannot be said to definitely have a disease-modifying effect" (Robottom 2011). Similar to α-tocopherol, neither lazabemide nor rasagiline, the two selective MAO-B inhibitors used in PD, are also devoid of the CAE effect of DEP (Miklya 2014).

Since the mid-1980s, further analysis of the characteristic enhancement of the catecholaminergic brain machinery in DEP-treated rats rendered probable that this effect is unrelated to the selective inhibition of MAO-B. The development of 1-phenyl-2-propylaminopental (PPAP), the DEP-analog devoid of a MAO inhibitory property, and an equally active stimulant of the catecholaminergic neurons as DEP, verified this suggestion (Knoll 1992). The first study which demonstrated that multiple, low dose administration of DEP enhances catecholaminergic activity in the brain and this effect is unrelated to MAO-B inhibition allowed for the discovery of the enhancer sensitive brain regulations (Knoll and Miklya 1994). P-phenylethylamine (PEA) and its best known synthetic derivatives (amphetamine and methamphetamine) are strong releasers of catecholamines from their plasmatic pools. Since the catecholamine releasing effect conceals the detectability of the enhancer-sensitive nature of the catecholaminergic neurons (Knoll 2016, Fig. 8), DEP's primary physiological function as a natural enhancer substance, as well as the fact that amphetamine and methamphetamine are, like DEP, PEA-derived synthetic enhancer substances, remained unknown.

The later realization that tryptamine is, like PEA, a natural enhancer (Knoll 1994) signaled the elaboration of BPAP as the most selective and potent synthetic enhancer substance currently known (Knoll et al. 1999).

Since in 1989, when the DATATOP study was performed, we all were convinced that the selective inhibition of MAO-B in the brain is responsible for DEP's unique pharmacological effects, but our further studies disaffirmed this view. I hope that clinicians will seriously consider that DEP is a PEA-derived synthetic enhancer substance which exerts its specific enhancer effect in a very low dose (0.001 mg/kg) (Knoll 1998); and the development of BPAP, the trypamine-derived, selective and most potent synthetic enhancer (Knoll et al. 1999) confirmed the importance of this new line in brain research. Furthermore, the proof in our recent longevity study that compared the low dose of the synthetic enhancers with their specific enhancer effect, and very high doses of DEP (0.25 mg/kg) and BPAP (0.05 mg/kg) exert their *non-specific enhancer effect* and prolong the life of rats is, from a practical point of view, important information. Since 0.25 mg/kg DEP is the peak dose in rats and 10 mg DEP daily is the optimum dose in patients to selectively block MAO-B activity in the brain and the same dose of DEP is also the peak dose with the non-specific enhancer effect, it remains for the future to exactly verify the participation of the non-specific enhancer effect and MAO-B inhibition in patients treated with 10 mg DEP daily.

Thank you Dr. Warnes for your valuable comments. I fully agree with you that the Reeve et al. review is an illuminating study which convincingly clarifies the relationship between aging and PD. I appreciate that you already tested the DEP transdermal patches and found them effective in a group of depressive patients.

Since DEP is still the only synthetic enhancer in clinical use, it remains for the future to finally test the already available synthetic enhancers, like PPAP and BPAP, in patients. BPAP, an extremely safe compound, proved to be in animal experiments the most effective synthetic enhancer substance. Since we recently detected that a previously unknown enhancer-sensitive tumor-manifestation-suppressing (TMS) regulation works in the rat brain (Knoll et al. 2017), the selection of a pilot group of patients with *de novo* diagnosed malignant tumor to test the appearance of the dramatic TMS effect of BPAP treatment observed in rats could be a reasonable first approach to appraise the significance of BPAP in suppressing the manifestation of malignant tumors.

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May 3, 2018

Hector Warnes' final response (4) to Joseph Knoll's additional response (2) to Hector Warnes' response (2)

I was privileged to maintain an ongoing debate with Professor Knoll on his scientific life's work particularly since the early 1960s when he discovered deprenyl, a phenylethyl- derivative with catecholaminergic activity.

Professor Thomas Ban conducted a superb interview of Professor Knoll which appeared in the Oral History of Neuropsychopharmacology Vol. 3, 2011. Professor Ban recently told me that Professor Knoll had died. I felt it was an irrecuperable loss of a great man whom I revered.

Joseph Knoll was born in 1925. He was Head of the Department of Pharmacology at the Semmelweis University of Medicine in Budapest from 1962 to 1992 when he retired as Emeritus.

I don't know what it took to survive the Nazi Holocaust. Professor Knoll, like Bruno Bettelheim, were the few exceptions of those who, not for lack of resilience or vulnerability, survivor guilt or perhaps sheer luck, were able to keep on living (life in death or death in life) in spite of all the odds against them.

Each of the two after the end of the war took a different path, Bettelheim along the path of psychoanalysis and Knoll along the path of exact experimental neurosciences and neuropharmacology. I don't really know what happens to those survivors when they lose

autonomy and waves of grief assault their memories without pity and when their body keeps on deteriorating into an inexorable end.

Bettelheim committed suicide at 86.

I only know that Professor Knoll was a very cultured man besides being a remarkable scientist and clinician. He told Professor Ban that in his youth he loved poetry and could recite up to 200 poems.

The epistolar dialogue I was privileged to have with Professor Knoll reminded me of Martin Buber I-Thou relationship because he had that extraordinary PRESENCE and an unusual capacity to listen and to share. I think of Bettleheim's ideas of a mass society: bureaucratized, technological, alienated, in the grips of aloneness and ruthless materialism. It is not unlike the I-It relationships described by Martin Buber based on detachment from others and filled with a utilitarian approach.

Contrariwise, in the I-Thou (*Ich-Du*) relationship one turns toward the other with openness and ethical respect. The dialogue underlines the presence of the other and the reciprocal capacity to listen and to share.

I felt that I had an unfinished dialogue with Professor Knoll and that behind his great life's work he was searching for connectedness and timelessness. In fact, much of his research had to do directly or indirectly with the mystery of our life-span.

August 30, 2018

April 2, 2020