Joseph Knoll: Enhancer Sensitive Brain Regulations and Synthetic Enhancers (Selegiline, BPAP) Which Counteract the Regressive Effects of Brain Aging

Chapter 5

Longevity studies with DEP and BPAP

As already recalled, the selective B type monoamine oxidase (MAO-B) inhibitory effect of selegiline/(-)-deprenyl (DEP)was for several years at the center of our interest and delayed the discovery of the drug's enhancer effect. Getting acquainted with the peculiar pharmacological spectrum of DEP, it was reasonable to put forward the concept that the progressive decrease in brain catecholamines and the trace amines is an unavoidable biochemical lesion of aging, which can be counteracted by appropriate continuous medication (Knoll 1982).

The discovery of the bell-shaped concentration/effect curve of the enhancer substances, in the low pico/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 concluded 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 three longitudinal studies performed on rats (Knoll 1988; Knoll and Miklla 2016; Knoll et al. 1989, 1994).

In the years when we performed our first and second longevity studies and worked with the robust Wistar-Loganrats (first generation of Wistar males x Logan females), we observed that the males which completed their second year of 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. 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 un-ability to ejaculate at an average of 112±9 weeks. Due to the anti-aging effect of DEP, rats injected subcutaneously,3-times a week with 0.25 mg/kg DEP, reached that stage at an average of 150±12 weeks (Knoll 1993).

THE LONGEVITY STUDIES PERFORMED WITH THE MAO-B INHIBITORY DOSE (0.25 mg/kg) OF DEP

In our first longevity study we worked with 132 sexually inexperienced 2-year-old males. We tested their copulatory 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 DEP, 3-times a week, and observed their behavioral performances to the end of their life.

In 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 (Knoll 1988, Table VI). DEP treatment prolonged 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 special 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 (Chapter 4), is responsible for the lifespan-prolonging effect. Thus, the brain engine works in the 2-year-old non-copulators on a lower activity level than in the 2-year-old sluggish males.

Results of the first longevity study, and the analysis of the peculiar physiological role of the catecholaminergic neurons to keep the brain in a continuously active state, as described in 1994 (Knoll 1994), determined the planning of the second longevity study.

As analyzed in detail in Chapter 4, the hypothesis out-lined in 1994 (Knoll 1994) suggested 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 low performing, shorter-living peer. To simplify this concept, we may say, that a better brain engine allows for a better performance and a longer lifespan.

Thus the concept clearly predicted 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 of the duration of life beyond the technical lifespan

(TLS), with a yet unpredictable upper limit, must be possible in all mammals, including the human species.

To test this concept we decided to perform our second longevity study on younger rats. We selected from a huge population of Wistar-Logan male rats the ***non-copulators*** *and the* ***sexually most active ones****. We measured their sexual potency and learning ability until the end of their life, and treated 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 sexually lowest performing (LP) and the latter for the highest performing (HP) rats. It is obvious that my main aim with the second longevity study was to test DEP-treatment's expected ability to transform an LP rat into a higher performing one.

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/kgDEP, dissolved in 0.9% NaCl and given in the same volume, 3-times a week, until the end of their life.

Out of the **94** LP animals, 46 were saline-treated. Out of the **99** HP animals, 49 were saline-treated. The mating and learning performances of saline-treated LP and HP rats were tested during a period of 108 weeks. Copulatory activity was tested once a week.

The learning performance of the rats was tested in the shuttle box. The rats were trained for a five-day period once in three months 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 lifespan between LP and HP rats.

The salt-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 salt-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 PERFORMANCE OF BOTH LP AND HP RATS AND SIGNIFICANTLY PROLONGED THEIR LIFESPAN

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 salt-treated peers and as long as the salt-treated HP rats. The DEP-treated HP rats (n=50) were sexually much more active than their salt-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 conditioned avoidance responses (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 salt-treated peers and out of the 50 rats 17 lived longer than the estimated technical lifespan. Considering the unique dose-related effect of an enhancer substance, we assume that out of the 1,600 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 (1,407 rats) falls between these two extremes.

THE SECOND LONGEVITY STUDY VERIFIED THAT LIFE-LONG DEP-TREATMENT TRANSFORMS OF A LOWER-PERFORMING, SHORTER LIVING RAT, INTO A BETTER-PERFORMING, LONGER-LIVING ONE

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 (Knoll 2005, Sect.1.3 and 4.2). 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 in the optimum concentration. Thus regarding the measured function we may look upon these two rats as the most talented in the tested population.

There is a gleam of hope that better understanding of the enhancer regulation in the cortical neurons may finally allow to define on a molecular level the physiological mechanism responsible of "man of talent"/"genius". As analyzed and discussed in detail in my monograph (Knoll 2005), since the natural endowments of the healthy human brain are identical, everybody is born with 100 billion neurons and 10^{11} bit capacity, *everybody has necessarily brilliant abilities which remain unexplored, unutilized.*

As it will be discussed in detail, 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 activity returns to the pre-weaning level. Developmental longevity, the happy, uphill period of life, is a short phase between weaning and sexual maturity (Knoll and Miklya 1995).

As it will be shown in Chapter 7, sexual hormones dampen the intensified enhancer regulation in the catecholaminergic and serotonergic neurons and activity returns to the pre-weaning level. This is the transition from adolescence to adulthood. The post-developmental/downhill period of life begins with sexual maturity and lasts until natural death (Knoll et al. 2000).

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 with the passing of time plays a crucial role in the behavioral consequences, the regressive effects of brain aging.

In this context our early work with DEP, prior to the discovery of the enhancer effect, already called our attention to the peculiar nature of DEP-induced facilitation of striatal dopaminergic neurotransmission. We studied in detail the phenomenon that striata of rats treated with 0.25 mg/kg DEP daily for at least three weeks released significantly more DA in the resting state and in response to KCl stimulation than the striata of rats treated daily with 0.1 mg/ 100 g salt solution. The finding that DEP increases the firing rate of the nigrostriatal dopaminergic neurons was in complete agreement with previous data, proving the increased turnover rate of DA in the striatum of male rats treated for 2-4 weeks with a daily dose of 0.25

mg/kg DEP. *The facilitation of striatal dopaminergic neurotransmission by long term treatment was highly specific.* The turnover rate of NE was decreased and the level of this amine remained unchanged in the brain stem of male rats; and no change in the turnover rate of SE was detected (Knoll 1993).

S*ince, as it will be shown in Chapter 8,the aging-related continuous slow decay of the natural enhancers result in the regressive effects of brain-aging, but the enhancer-sensitive neurons do not age, and since we already developed proper synthetic enhancers, we are able to counteract brain aging.*

The daily preventive administration of a synthetic enhancer substance from sexual maturity until death can maintain the activity of the catecholaminergic and serotonergic neurons on a higher activity level, and we can already slow aging-related decay of physical and mental welfare. *From practical point of view this is an essential recent message of our work* (Knoll and Miklya 2016).

The convincing experimental evidence that the 0.25 mg/kg dose of DEP, the peak dose which inhibits MAO-B in the brain is also the peak dose with the non-specific enhancer effect and the increasing amount of data in support that the enhancer effect of DEP and (2*R*)-1-(1-benzofuran-2-yl)-*N*-propylpentane-2-amine (BPAP) is primarily responsible for the beneficial pharmacological effects of the synthetic enhancers initiated our recently published longevity study with low doses of DEP and BPAP (Knoll and Miklya 2016).

The discovery of the enhancer regulation in the mammalian brain and the study of the catecholaminergic and serotonergic neurons as enhancer-sensitive brain regulations (Knoll 1994, 2005, 2016), the identification of β-phenylethylamine (PEA) and tryptamine as natural enhancer substances (Knoll 1994, Knoll et al. 1996a, 1999), the proof that DEP is only PEA-derived synthetic enhancer substance devoid of the catecholamine releasing property (Abdorubo and Knoll 1988) revealed that PEA and amphetamines act as catecholaminergic activity enhancer (CAE) substances and release catecholamines from the plasmaticpools only in high concentrations (Knoll 1998). *Since the continued catecholamine-releasing effect of PEA and amphetamines concealed their CAE effect (Knoll 2016, Fig.8), PEA's natural enhancer function remained until the development of DEP undetected.* The development of (BPAP), the tryptamine-derived*selective* synthetic enhancer substance (Knoll et al. 1999), confirmed that the enhancer-sensitive brain regulations represent a promising new brain-research domain (Knoll 1992, 2012;Knoll et al. 2002;Miklya 2016).

Taking all this in consideration we decided to perform the third longevity study, with the aimto clarify the role of the enhancer effect in life extension (Knoll and Miklya 2016) *and to confirm that the enhancer sensitive neurons do not age.*

PROOF THAT THE SPECIFIC ENHANCER DOSES OF DEP AND BPAP ARE RESPONSIBLE FOR LIFE EXTENSION

We performed the third longevity study on male Wistar rats with selected peak doses of DEP and BPAP, in which the synthetic enhancers exerted their specific and non-specific enhancer effects, and demonstrated that the enhancer effect of DEP and BPAP are responsible for life extension. We treated rats three-times in a week with 0.0001 mg/kg BPAPaiming to test their learning ability in the shuttle box as they completed their 18th month of age, in order to demonstrate that BPAP-treatment prevents the enhancer-sensitive dopaminergic neurons from aging.

The longevity study demonstrated that the enhancer effect of DEP and BPAP are responsible for life extension (Knoll and Miklya 2016)*, and presented also the first experimental evidence that life-long treatment of rats with0.0001 mg/kg BPAP counteracts brain aging*.

As shown in before, DEP, *the unique PEA derivative free of the catecholamine releasing-property,* was the first experimental tool which enabled the revelation of the enhancer regulation in the mammalian brain. BPAP, devoid of MAO-B inhibitory effect, is at present the available most selective and potent synthetic, tryptamine-derived enhancer substance. We tested during the last decades the catecholaminergic and serotonergic neurons as potential enhancer sensitive ones. DEP is a CAE substance, poorly acting on the serotonergic neurons. BPAP is even as a CAE substance much more potent than DEP and is an even more potent enhancer of the serotonergic neurons.

It is well known that DEP significantly prolongs the life of rats (Bickford et al. 1997; Dallo and Koles 1996; Kitani et al. 1993; 2002, 2005; Knoll 1988, Knoll et al. 1989, 1994, Milgram et al. 1990,), mice (Freisleben et al. 1994, Archer and Harrison 1996), Syrian hamsters (Stoll et al. 1997), beagle dogs (Ruehl et al. 1995), and acts even on Drosophila melanogaster (Jordens et al. 1999). The first two longevity studies (Knoll 1988, Knoll et al. 1989, 1994) were performed with 0.25 mg/kg DEP, the MAO-B inhibitory peak dose of DEP. The Knoll and Miklya (2016) study was the first performed with low doses of DEP (0.001 and 0.1

mg/kg) and BPAP (0.0001 and 0.05 mg/kg) acting as peak doses eliciting the specific and non-specific enhancer effect, respectively.

The longevity study was performed with 200 male Wistar rats. Ten-week-old rats were randomly assigned into five groups and treated three-times a week (Monday, Wednesday, and Friday), subcutaneously, with saline, DEP and BPAP, respectively, until their natural death, as shown below:

Group	Treatment	Dose	N
1.	Saline	0.05 ml/100 g	40
2.	DEP	0.1 mg/kg	40
3.	DEP	0.001 mg/kg	40
4.	BPAP	0.05 mg/kg	40
5.	BPAP	0.0001 mg/kg	40

To select the optimal low doses of DEP and BPAP for the longevity study we used a modified version of the shuttle box, originally described in 1966 (Bovet et al. 1966). The acquisition of a two-way conditioned avoidance reflex (CAR) was analyzed during 5 consecutive days. The rat was placed in a box divided inside into two parts by a barrier with a small gate in the middle, and the animal was trained to cross the barrier under the influence of a conditioned stimulus (CS, light flash). If it failed to respond within 5s, it was trained with a foot-shock (1mA), the unconditioned stimulus (US). If the rat failed to respond within 5s to the US, it was classified as an escape failure (EF). One trial consisted of 10s inter-trial interval, followed by 20s CS. The last 5s of CS overlapped the 5s US. Rats received 100 avoidance trials / day. At each learning session, the number of CARs, EFs and inter-signal reactions (IRs) were automatically counted.

As shown in Chapters 2 and 3, the bi-modal, bell-shaped concentration effect curve is characteristic to the CAE effect of the enhancer substances. We first took notice of this peculiar behavior in the course of our first experiments when we realized the CAE effect of DEP (Knoll 2005). Nevertheless, only the precise analysis of BPAP's enhancer effect, the selective and today's most potent enhancer substance, rendered the unquestionable distinction between the *specific* and *non-specific* enhancer effect possible. The bi-polar, bell-shaped

nature of the enhancer effect was confirmed on cultured rat hippocampal neurons (Knoll et al. 1999); and exactly analyzed on isolated locus coeruleus (Knoll et al. 2002).

To select the optimal *specific* and *non-specific* enhancer doses of DEP and BPAP for our longevity study, we analyzed the acquisition of a two-way conditioned avoidance reflex (CAR) in a shuttle box, using tetrabenazine (TBZ)-treatment. TBZ (1 mg/kg sc.) reversibly blocks the vesicular monoamine transporter 2 (VMAT2) and within 1 hour depletes at least 90% of NE and DA from their transmitter-stores in the nerve terminals of the catecholaminergic neurons in the brain stem (Scherman et al. 1983). As quoted by Schreiber et al. (1999) "noradrenergic neurotransmission – that is, neuronal noradrenaline depletion – can therefore be postulated to form one major origin of TBZ induced depression. In line with this assumption brain specific CAEs such as phenylethylaminehave been shown to antagonize TBZ-induced depression-like behavior in rats (Knoll et al. 1996b)". Due to the weak performance of the catecholaminergic brain engine, the activation of the cortical neurons remains below the required level for the acquisition of a CAR. According to our experience (we studied for years the drug families worth trying), TBZ-induced inhibition of learning performance can only be antagonized by administration of a synthetic CAE substance or by the complete inhibition of MAO-A, whereas selective inhibition of MAO-B or inhibition of the reuptake of catecholamines and/or SE is ineffective (Knoll et al. 1992). F

DEP, the first selective inhibitor of MAO-B, is classified since the 1970s in all papers and text books as the reference compound to block this enzyme. DEP's CAE effect, the significance of which was realized only in the late 1990s, is still waiting to be controlled and pass into common knowledge.

Figure5.1 demonstrates that DEP exerts its enhancer effect in the bi-modal, bell-shaped manner characteristic to the CAE substances. It is of extreme importance that the dose of DEP which fully blocks MAO-B is also the optimum dose which elicits the non-specific CAE effect. Since rasagiline and lazabemide are devoid of the enhancer effect (Miklya 2011), it remains for the future to find out the role of the CAE effect in the therapeutic benefits observed for decades.

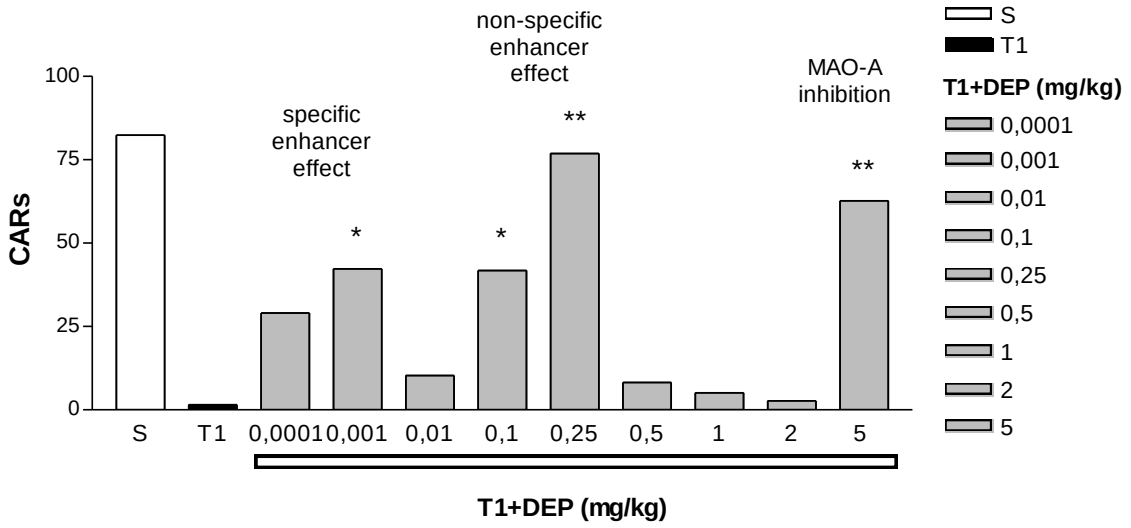

Figure 5.1. *Selection of optimal doses of DEP for the longevity study in the shuttle box.* Antagonism of TBZ-induced inhibition of learning performance in the shuttle box on the fifth day by DEP in the bi-modal, bell-shaped manner characteristic to the CAE substances. Measured: (S) the ability of saline-treated (control) rats to fix conditioned avoidance responses (CARs); (T1) the inhibition of the learning ability of rats treated subcutaneously with 1 mg/kg TBZ, one hour prior to training; (T1 + DEP) the ability of DEP to antagonize in a dose related manner the inhibitory effect of TBZ. Significance in the performance between the groups was evaluated by one-way ANOVA: followed by Newman-Keuls multiple comparison test. *$p<0.05$; **$p<0.01$

A bi-modal, bell-shaped concentration effect curve is also characteristic to the enhancer effect of BPAP. Figure5.2 shows that 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). BPAP acts only in a very high concentration on MAO-A (Fig. 5.2), and is devoid of MAO-B inhibitory potency. DEP, as shown in Fig. 5.1, is a much less potent CAE substance than BPAP, but otherwise it exerts its *specific* and *non-specific* enhancer effect with the same characteristics as BPAP.

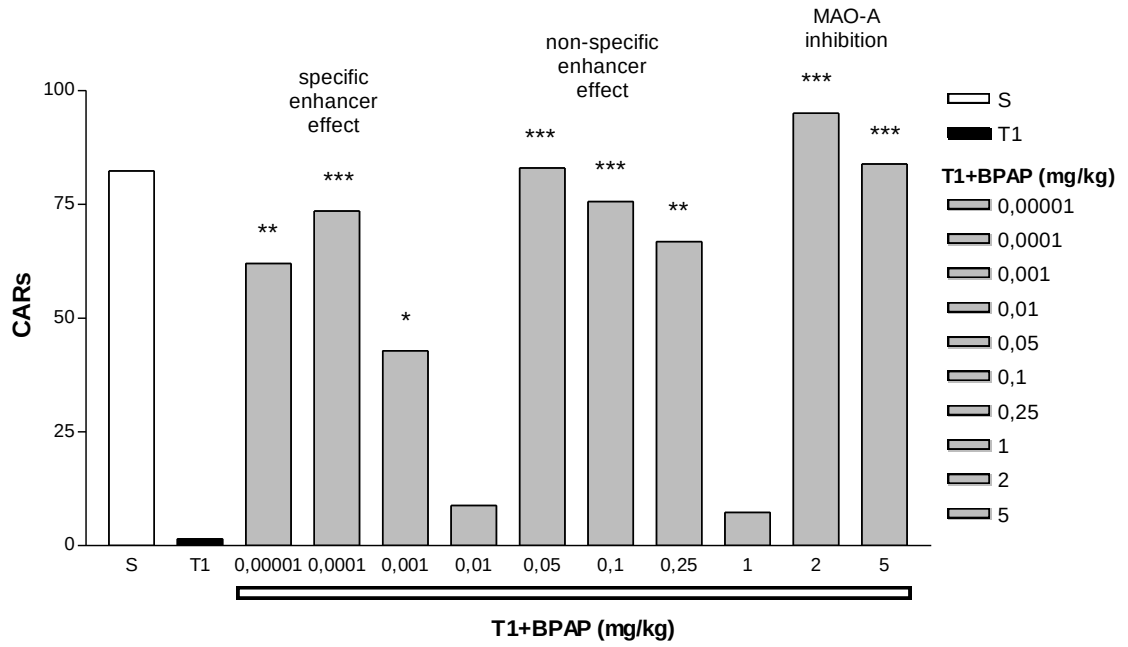

Figure 5.2. *Selection of optimal doses of BPAP for the longevity study in the shuttle box.* Antagonism of TBZ-induced inhibition of learning performance in the shuttle box on the fifth day by BPAP in the bi-modal, bell-shaped manner characteristic to the CAE substances. Measured: (S) the ability of 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 TBZ, one hour prior to training; (T1 + BPAP) the ability of BPAP to antagonize in a dose related manner the inhibitory effect of TBZ. Significance in the performance between the groups was evaluated by one-way ANOVA: followed by Newman-Keuls multiple comparison test. *p<0.01; **p<0.001, ***p<0.0001

The longevity study with low doses of DEP and BPAP

Table 5.1. The first death (D)in the group treated with saline or an enhancer substance

Age of rats (months)	Saline 0.5 ml/kg	DEP 0.1 mg/kg	DEP 0.001mg/kg	BPAP 0.05 mg/kg	BPAP 0.0001 mg/kg
9	D				
11		D			
13			D		
14				D	
16					D

Table 5.1 shows the first rat death (D) in groups of rats treated with saline or with an enhancer substance thus immediately suggesting the beneficial influence of BPAP on the lifespan of rats. The first rat died 7 months later in the group of rats treated with 0.0001 mg/kg BPAP than the first rat in saline-treated group. BPAP is known to be a more potent enhancer substance than DEP (Knoll 2005) and acts in this test too accordingly

Table 5.2. The average lifespan of rats treated with saline, DEP and BPAP, respectively

TREATMENT	**AVERAGE LIFESPAN IN WEEKS**	
Saline	94.23 ± 3.48	[100%]
DEP 0.1 mg/kg	105.20 ± 3.07*	[112%]
DEP 0.001 mg/kg	101.60 ± 3.38	[108%]
BPAP 0.05 mg/kg	107.00 ± 3.45**	[114%]
BPAP 0.0001 mg/kg	107.00 ± 3.14***	[114%]

Student t-Statistic for two-means, *P<0.05; **P<0.02; ***P<0.01, mean±S.E.M., N=40. One-way ANOVA F(4/195)=2.632, P=0.0356 followed by Dunnet's multiple comparison test saline versus BPAP 0.05 and BPAP 0.0001 *p<0.05.

Table5.2 shows average lifespan of rats treated with saline DEP and BPAP, respectively. Due probably to the low dose treatment with DEP only three times a week, the specific dose of DEP (0.001 mg/kg) did not prolong lifespan significantly.

Table 5.3. Shortest and longest living rats treated with saline, DEP and BPAP, respectively.

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%]	135 [100%]
BPAP 0.05 mg/kg	59 [164%]	153 [113%]
BPAP 0.0001 mg/kg	65 [180%]	145 [107%]

Table 5.3 compares the lifespan of the shortest and longest living rat in groups treated with saline, DEP and BPAP, respectively. The differences are remarkable. For example, in the group of rats treated with 0.05 mg/kg BPAP, the shortest living rat lived 59 weeks, 23 weeks (1.64 times) longer than its saline-treated peer (36 weeks). The longest living rat lived 153 weeks, 18 weeks longer than its saline-treated peer (135 weeks). In the group of rats treated with the extremely low dose of BPAP (0.0001 mg/kg), the shortest living rat lived 65 weeks, 1.8 times longer than its saline-treated peer (36 weeks) and the longest living rat in this group lived 145 weeks, 10 weeks longer than its saline-treated peer (135 weeks).

In the saline-treated group only 14 rats, in the group treated with 0.1 mg/kg DEP 22 rats, in the group treated with 0.001 mg/kg DEP 19 rats, in the group treated with 0.05 mg/kg BPAP 22 rats, and in the group treated with 0.0001 mg/kg BPAP 23 rats lived longer than 2-years.

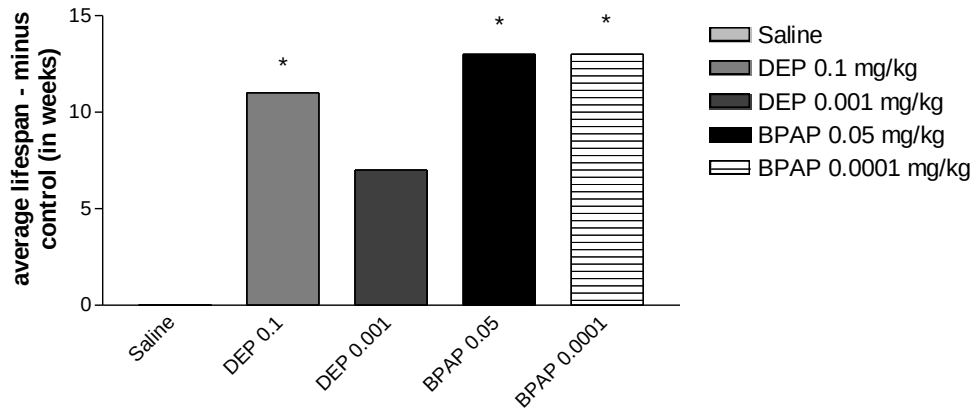

Figure 5.3. The average lifespan of rats minus control in weeks. One-way ANOVA: $F(4/195)=2.0356$ $*p<0.05$

Figure 5.3 shows the average lifespan of rats minus control in weeks. The group of rats treated with 0.1 mg/kg DEP, 0.05 mg/kg and 0.0001 mg/kg BPAP lived significantly longer than their saline-treated peers. Figure 5.3 demonstrates that though the change was not statistically significant, even the rats treated with 0.001 mg/kg DEP lived longer than their saline-treated peers.

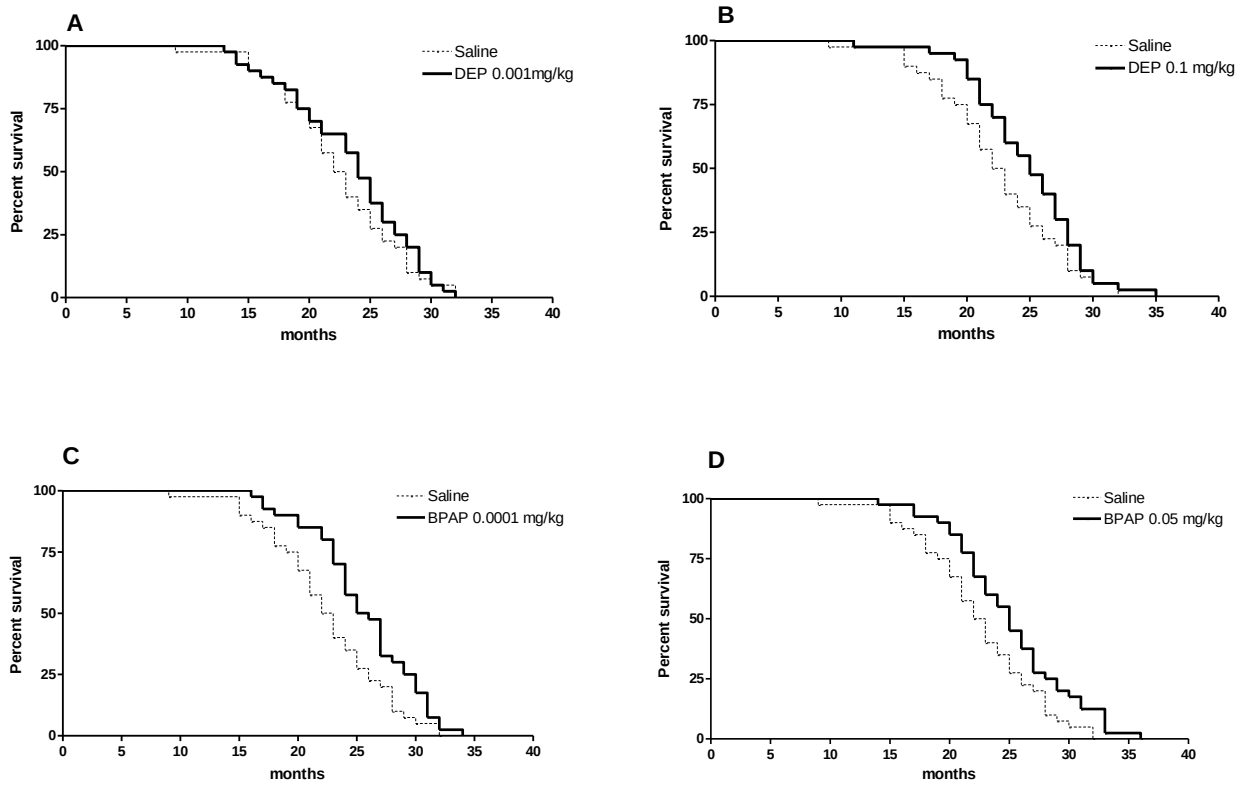

Figure 5.4. Life extension due to a low dose treatment with enhancer substances. Treatment with saline versus DEP (A, B) and BPAP (C, D) in doses selected in the shuttle box test for the longevity study. Kaplan-Meier test, A: DEP 0.001 mg/kg p=0.434 (ns), B: DEP 0.1 mg/kg p=0.0866 (ns); C: BPAP 0.0001 mg/kg *p<0.02 (P=0.011); D: BPAP 0.05 mg/kg *p<0.02 (P=0.011)

Further studies revealed that since aging of the natural enhancers is responsible for the regressive effects of brain aging, but the enhancer-sensitive neurons do not age, the life-long treatment of rats from sexual maturity with 0.0001 mg/kg BPAP prevents the aging-related decay of the dopaminergic neurons, which we used as the first model to present unequivocal experimental evidence that the enhancer-sensitive neurons remain sensitive toward BPAP, the selective and highly potent synthetic enhancer.

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