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Longevity study with low doses of selegiline/(-)-deprenyl and (2R)-1-(1-benzofuran-2-yl)-N-propylpentane-2-amine (BPAP)

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Abstract

Aims: The first longevity study demonstrating that rats treated with the MAO-B inhibitory dose of (-)-deprenyl (0.25 mg/kg) lived significantly longer than their saline-treated peers was published in 1988, and corroborated in many papers. The recent findings that (-)-deprenyl is primarily a PEA-derived synthetic catecholaminergic activity enhancer substance; (2R)-1-(1-benzofuran-2-yl)-N-propylpentane-2-amine (BPAP) is a tryptamine-derived synthetic enhancer substance, initiated our first longevity study on rats with low enhancer doses of (-)-deprenyl and BPAP to test the enhancer effect's role in life extension.

Main methods: We used the shuttle box technique for selecting the optimum doses of (-)-deprenyl and BPAP. (-)-Deprenyl exerts in rats in 0.001 mg/kg its 'specific' enhancer effect and in 0.1 mg/kg its 'non-specific' enhancer effect. BPAP exerts its 'specific' enhancer effect in 0.0001 mg/kg and its 'non-specific' enhancer effect in 0.05 mg/kg. Groups of male Wistar rats (N=40) were treated subcutaneously from their 10^{th} week until death, three times weekly, with saline (0.5 ml/kg), and the selected doses of (-)-deprenyl or BPAP, respectively. As an indicator of aging we tested the age-related changes in their learning ability.

Key findings: Rats treated with 0.0001 or 0.05 mg/kg BPAP lived significantly longer than their saline treated peers ($P<0.02$) and BPAP was more potent in extending rats' lifespan than (-)-deprenyl. 18-month-old rats treated with 0.0001 mg/kg BPAP were as good learners as 3-month-old saline treated rats.

Significance: The study revealed that the enhancer effect is responsible for life extension.

Key words: selegiline/(-)-deprenyl, (2R)-1-(1-benzofuran-2-yl)-N-propylpentane-2-amine (BPAP), aging, lifespan, longevity study, low dose treatment, enhancer substances

Introduction

The discovery of the enhancer regulation in the mammalian brain and the study of the catecholaminergic and serotonergic neurons as enhancer sensitive brain regulations [1-3], the identification of β-phenylethylamine (PEA) and tryptamine as natural enhancer substances [4], the proof that selegiline/(-)-deprenyl (DEP) is the first PEA-derived synthetic enhancer substance devoid of the catecholamine releasing property [5], and the development of (2R)-1-(1-benzofuran-2-yl)-N-propylpentane-2-amine (BPAP), a tryptamine-derived, most potent synthetic enhancer substance [6], allowed for a promising new brain-research domain [7-10].

The discovery of the enhancer-sensitive brain regulations and the development of DEP and BPAP, the first synthetic enhancer substances, explain the promptness of activation in life dangerous assault/escape situations. Cerebral dopaminergic neurons are primarily responsible for the general activation of the cortex. It is well known that regarding the excitability and function of the dopaminergic neurons, electrophysiological studies with rodents and primates have shown that these neurons are silent or spontaneously active [11]. It is a remarkable unique effect of the enhancer substances that they transform the silent dopaminergic neurons into spontaneously active entities [3].

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 catecholaminergic activity enhancer (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 [12-18], mice [19,20], Syrian hamsters [21], beagle dogs [22], acts even on Drosophila melanogaster [23]. The first longevity studies were performed with 0.25 mg/kg DEP. This study is the first performed with low, enhancer doses of DEP and BPAP.

Materials and Methods

Materials

DEP was supplied by Sanofi-Chinoin (Budapest, Hungary); BPAP by Fujimoto Pharmaceutical Company (Osaka, Japan); Tetrabenazine (TBZ) (synthetized by Prof. Csaba Szantay, Department of Organic Chemistry in the Technical University, Budapest, Hungary).

Longevity study with low doses of DEP and BPAP

The longevity study was performed with 200 male Wistar rats. 4 animals were housed in a Plexiglas cages (44x42x18 cm), temperature- (21±2°C) and humidity-controlled environment, and were maintained on 12:12hr light/dark cycle. All rats were weighted monthly and had food and water *ad libitum*. 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

Selection of the optimal low doses of DEP and BPAP for the longevity study using the shuttle box technique.

We used a modified version of the shuttle box, originally described in 1966 [24]. 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) are automatically counted.

A 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 [2]. 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 [6]; and exactly analyzed on isolated locus coeruleus [25].

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. TBZ-treatment (1 mg/kg sc.) reversibly blocks the vesicular monoamine transporter 2 (VMAT2) and within 1 hour depletes at least 90% of norepinephrine and dopamine from their transmitter-stores in the nerve terminals of the catecholaminergic neurons in the brain stem [26]. As quoted by Schreiber et al. [27] "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 catecholaminergic activity enhancers (CAEs) such as phenylethylamine have been shown to antagonize TBZ induced depression-like behavior in rats [4]". 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), 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 [28].

DEP, the first selective inhibitor of B-type MAO, is classified since the 1970s in all papers and textbooks as the reference compound to block this enzyme, and its CAE effect, the real significance of which was realized only in the late 1990s, has unfortunately remained neglected.

Measurement of the aging-related changes in the learning ability of rats

We tested 3 monthly in selected naïve group of rats treated with saline, DEP and BPAP, respectively, the aging-related changes in learning ability as significant measure of the anti-aging effect of the enhancer substances.

Statistical analysis

The shuttle box data were analyzed by two-way ANOVA followed by Bonferroni post-hoc test; and the fifth day results were calculated by one-way ANOVA followed by Newman-Keuls or Dunnet's multiple comparison test. For the longevity study we used Kaplan-Meier statistical test. Differences were considered significant at $p<0.05$.

Results

Selection of the optimal low doses of DEP and BPAP for the longevity study using the shuttle box technique.

Fig. 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 [29], it remains for the future to find out the role of the CAE effect in the therapeutic benefits observed for decades.

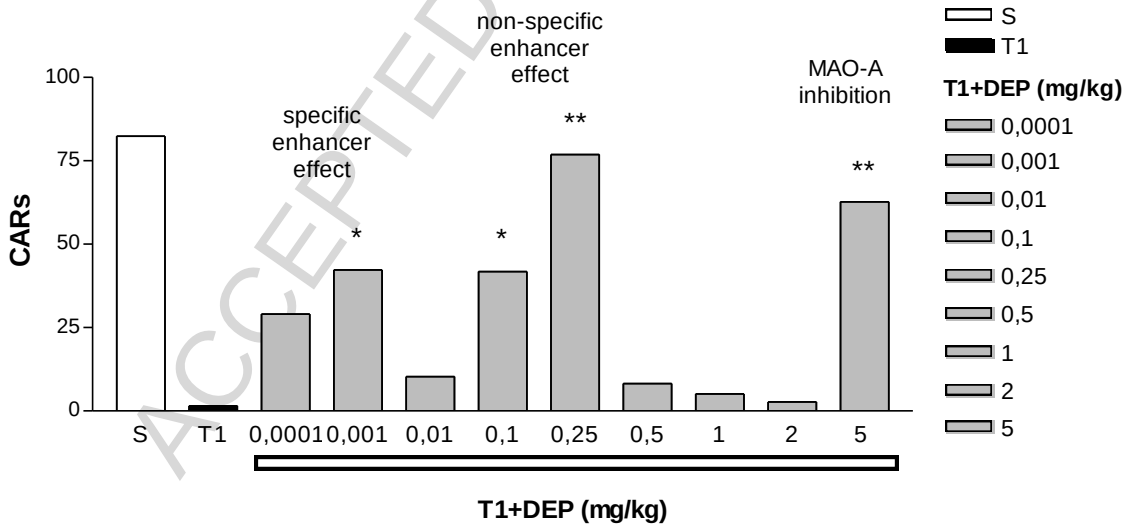

Figure 1. *Selection of optimal doses of DEP for the longevity study in the shuttle box.* Antagonism of tetrabenazine-induced inhibition of learning performance in the shuttle box on the fifth day by DEP in the bi-modal, bell-shaped manner characteristic to the CAE substances. Measured: (S) the ability 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 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 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. Fig. 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.2), and is devoid of MAO-B inhibitory potency. DEP, as shown in Fig.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.

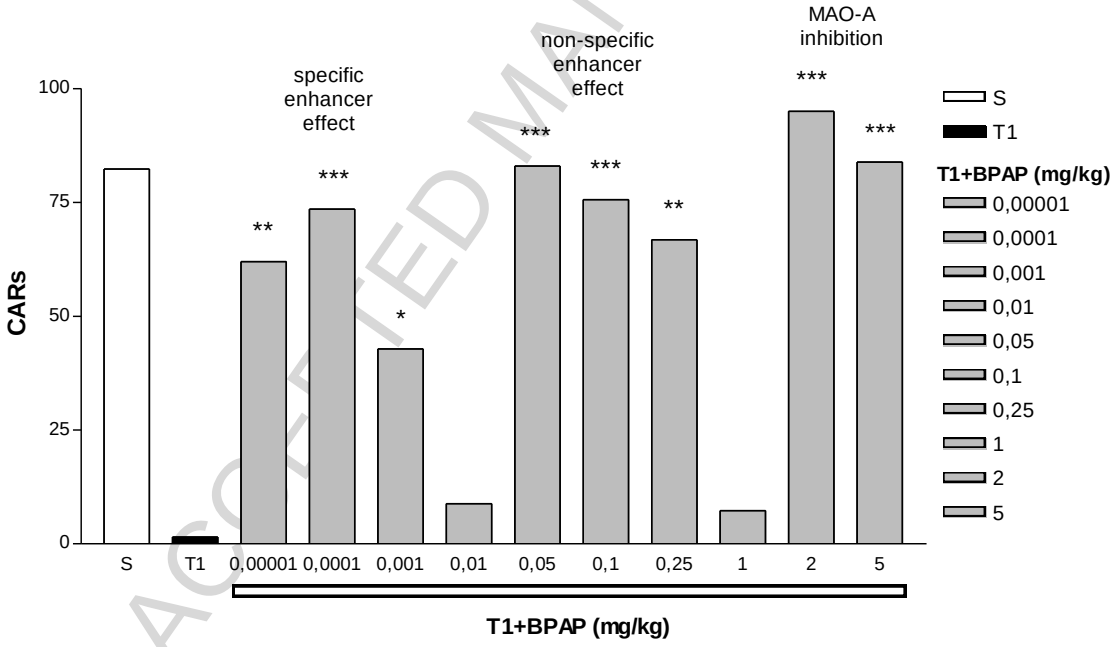

Figure 2. *Selection of optimal doses of BPAP for the longevity study in the shuttle box.* Antagonism of tetrabenazine-induced inhibition of learning performance in the shuttle box on the fifth day by BPAP in the bi-modal, bell-shaped manner characteristic to the CAE substances. Measured: (S) the ability 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 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 one-way ANOVA: followed by Newman-Keuls multiple comparison test. **P*<0.01; ***P*<0.001, ****P*<0.0001

Longevity study with low doses of DEP and BPAP

Table 1. The first death 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 1 shows the first rat death 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 [2] and acts in this test too accordingly.

Table 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.

Tables 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 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 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.

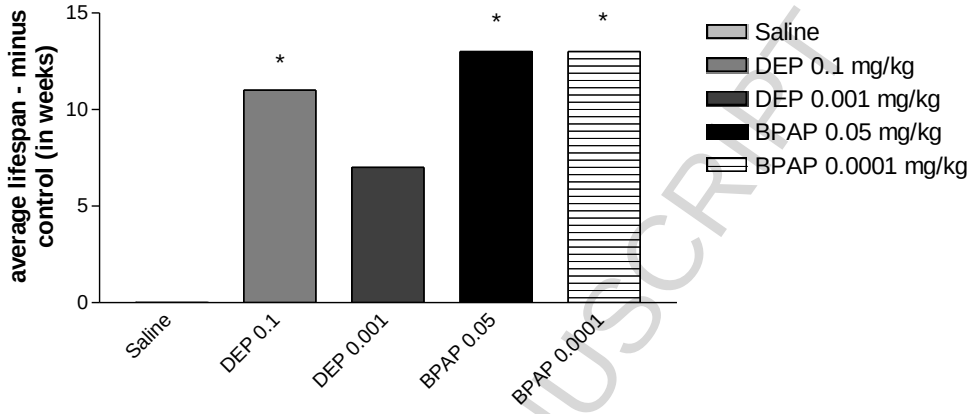

Figure 3. The average lifespan of rats minus control in weeks. One-way ANOVA: $F(4/195)=2.0356$ $*P<0.05$.

Fig.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. Fig.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.

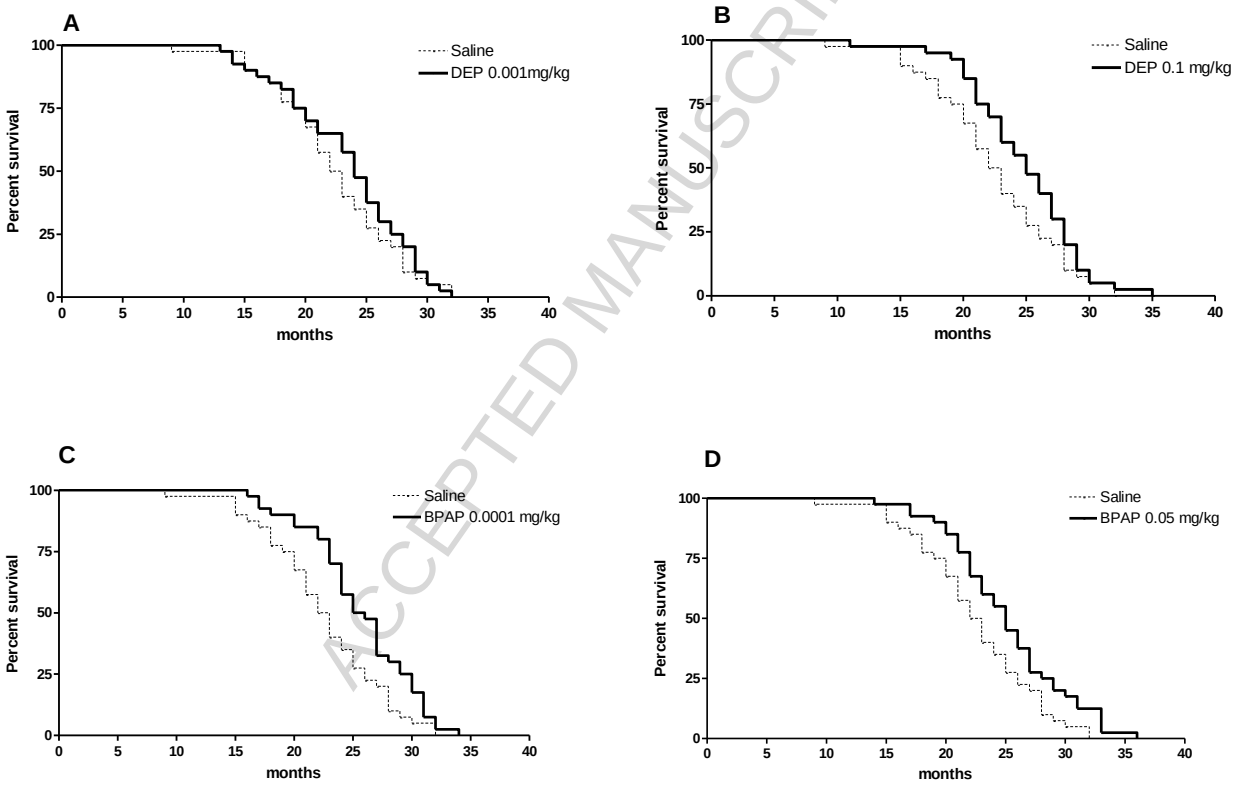

Figure 4. Life extension due to a low dose treatment with enhancer substances. Treatment with saline versus DEP (A, B) and BPAP (C, D) in doses selected in the shuttle box test for the longevity study (see Methods). Kaplan-Meier test, A: DEP 0.001 mg/kg P=0.434 (ns), B: DEP 0.1 mg/kg P=0.0866 (ns); C: BPAP 0.0001 mg/kg *P<0.02 (P=0.011); D: BPAP 0.05 mg/kg *P<0.02 (P=0.011)

Fig.4A compares the death rate of rats treated with saline or 0.001 mg/kg DEP, the peak dose with the 'specific' enhancer effect (see Methods: Fig.1). Fig.4B shows the death rate of rats treated with saline or 0.1 mg/kg DEP, the peak dose of the 'non-specific' enhancer effect. Calculated with the Kaplan-Meier test, neither 0.001 nor 0.1 mg/kg DEP-treatment significantly extended lifespan; the tendency of effectiveness seems, however, clear. In the longevity studies, rats treated three times a week with 0.25 mg/kg DEP lived significantly longer than their saline treated peers [13,14]. Since an exact study in the shuttle box revealed that 0.25 mg/kg is the peak dose of the 'non-specific' enhancer effect (see Methods: Fig.1), but 0.25 mg/kg DEP is also the peak dose which completely inhibits MAO-B activity in the brain, we performed the longevity study with 0.1 mg/kg dose of DEP which slightly inhibits MAO-B activity instead of previously used 0.25 mg/kg.

DEP is a non-selective, much less potent enhancer substance than BPAP. In contrast to DEP, three times per week treatment with either 0.0001 or 0.05 mg/kg BPAP was sufficient in achieving a significant prolongation of life (Fig.4C and D).

Measurement of the aging-related changes in the learning ability of rats

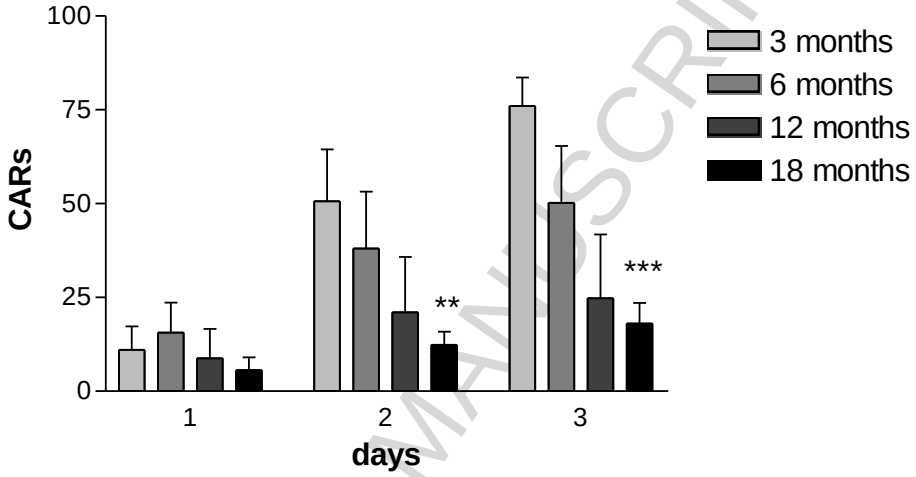

Figure 5. The age-related physiological decline in the learning ability of 3-, 6-, 12- and 18 month-old saline-treated rats. CARs-conditioned avoidance responses. Two-way ANOVA followed by Bonferroni post-hoc test: CAR age $F(3/48)=5.961$ **$p<0.01$; days $F(2/48)=8.746$ ***$P<0.001$. N=5

Fig.5 shows the conditioned avoidance responses in groups of 5 saline-treated rats selected from the running longevity study. The young, 3-month-old rats showed their normal performance; the 6 month-old group of rats showed already a considerable decline in their learning performance. The 12 month-old rats gave evidence of further loss in their ability to build conditioned avoidance responses, nevertheless the difference according to the Bonferroni post-hoc test is still not significant, obviously due to the small number of the tested rats (N=5). The learning ability of the 18 month-old rats changed already significantly.

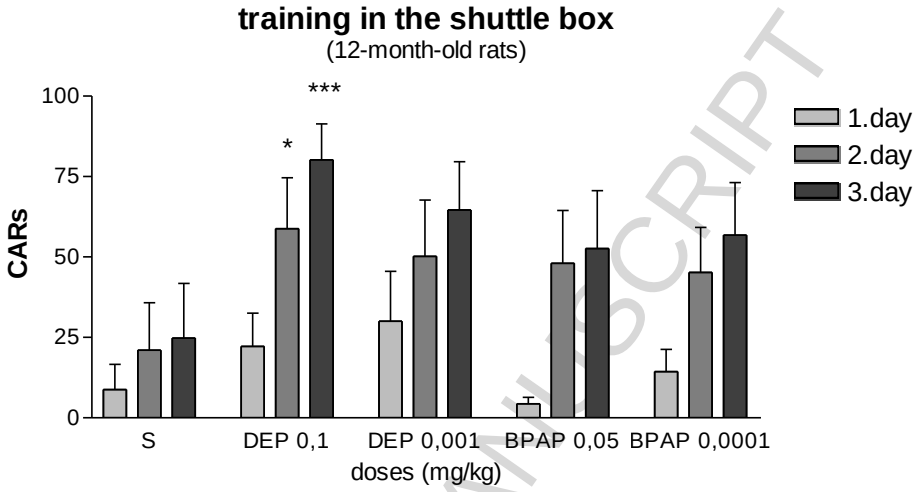

Figure 6. The age-related physiological decline in the learning ability of 12 month-old saline-treated rats compared to 12 month-old DEP- and BPAP-treated rats. CARs-conditioned avoidance responses. Two-way ANOVA: CAR treatment $F(2/60)=10.80$ ***$P<0.0001$; days $F(4/60)=2.881$ *$P<0.05$. N=5.

Fig.6 presents evidence that compared to the saline-treated 12-month-old rats the enhancer substances enhanced the learning ability in both specific and non-specific enhancer dosages but only 0.1 mg/kg DEP increased significantly the number of conditioned avoidance responses.

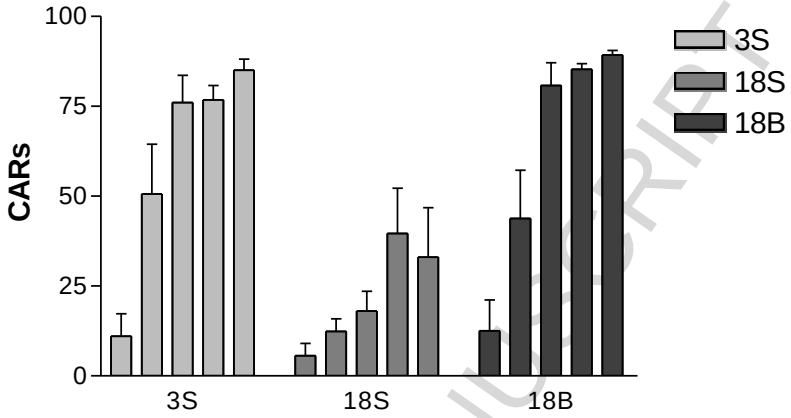

Figure 7.

3S: The full capacity of three month-old saline-treated rats in their ability to fix a condition avoidance response in the shuttle box during training for 5 consecutive days.
18S: The aging-related serious decline in the ability of saline-treated 18 month-old rats in building a condition avoidance response in the shuttle box during 5-day training.
18B: In contrast to saline-treated 18 month-old rats the longevity treatment with 0.0001mg/kg BPAP dramatically changed the ability of rats to fix in a 5 day consecutive training of a conditioned avoidance response. The performance of the18 month-old BPAP-treated rats was equivalent with the 3 month-old rat's performance. Two-way ANOVA; $F(8/55)=2.284$ $^{*}P<0.05$.

Fig.7 presents evidence that 18-month-old rats treated with 0.0001 mg/kg BPAP changed the learning ability of rats dramatically. The 18-month-old rats performed in the shuttle box like 3-month-old rats, clearly indicating that the highly potent enhancer substance transformed the silent catecholaminergic neurons into spontaneously active entities.

Discussion

In the 1950s, behavioral studies on rats proved the importance of the catecholaminergic brain machinery (the engine of the brain) in the fixation of acquired drives [30]. Although the amphetamines, releasers of catecholamines, the best compounds known in those days to stimulate the brain engine, were used, they unforeseeably disturbed purposeful behavior. To avoid this side effect, a structure-activity-relationship study was designed and performed with the aim to combine methamphetamine, the PEA-derivative, with newly developed MAOIs. Attaching the propargyl-group to methamphetamine to block MAO activity, DEP was finally selected for detailed analysis. The first publication in English appeared in 1965 [31].

DEP was originally designed as a new antidepressant. Varga showed first that DEP is a prompt acting antidepressant [32]. The finding was first confirmed by Mann and Gershon [33] and later in numerous papers. In 2002, Bodkin and Amsterdam published their first clinical trial with a new DEP preparation, the selegiline-transdermal-system (STS) [34]. In 2006 the FDA approved STS (Emsam) for depression.

In 1963, in the *Lancet*, a calamitous number of clinical reports gave accounts of patients treated with MAOIs that temporarily developed hypertensive crises. Blackwell suggested that the symptoms were associated with the ingestion of high amounts of tyramine in cheese, the metabolism of which is inhibited by the MAOIs ("cheese effect") [35]. This side effect restricted their clinical use. The discovery that DEP is a unique MAOI free of the "cheese effect" [36]; and this was verified in 1978 in human studies [37,38], was of notable therapeutic importance. The discovery that DEP is the unique MAOI free of the cheese effect made it possible to combine levodopa+DEP in PD and Birkmayer achieved a levodopa-sparing effect without signs of hypertensive reactions [39]. This study and a *Lancet* Editorial [40] initiated the world-wide use of DEP in PD.

The second highly important novelty in DEP's pharmacological spectrum, presented in 1971 at the First International MAO Symposium, was the finding that DEP is not simply one the MAOI's, but the first selective inhibitor of MAO-B [41]. The paper has become a citation classic. DEP soon achieved its place in research and therapy.

The last step 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 defined 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, and it was soon proved that this effect of DEP is unrelated to the inhibition of MAO-B [42,43].

Fig.7 clearly demonstrates the essence of the enhancer regulation in the mammalian brain. We see that the randomly selected young rats, treated for 1-month with 0.0001 mg/kg BPAP, easily built a conditioned avoidance response in the shuttle box during the five day period. However, due too normal aging, 18-month-old saline-treated rats were poor learners. Fig.7 demonstrates the dramatic difference in the learning ability of 18-month-old rats treated with 0.0001 mg/kg BPAP. We saw for the first time 18-month-old rats performing in the shuttle box like 3-month-old rats, due to BPAP–induced transformation of the silent enhancer sensitive catecholaminergic neurons into spontaneous firing entities.

It is worth reviewing 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 [3, p 12].

As shown in methods, the addition of 0.0001 mg/kg BPAP to 1 mg/kg tetrabenazine fully restored the learning ability of rats. Since BPAP-treatment fully restored the ability of the cortex to fix CAR's, there is no denying the fact that VMAT2 works again despite the presence of tetrabenazine. Thus a three-week daily treatment with a low dose of DEP or BPAP kept VMAT2 working on an enhanced activity level. However, also a modulation of VMAT2 activity cannot be excluded, as conformational changes or downstream regulatory mechanisms such as G proteins have not been yet analyzed.

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 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) [3, Table 10]. 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.

The presently known 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 in action the downhill period of life and the aging-related slow decay of the enhancer regulation continues until death [44,45]. It is obvious that 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.

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 permanently 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 [10].

Decades ago, based on the concept that the long term administration of DEP may improve the quality of life in the declining years [42], and this effect of DEP is unrelated to the inhibition of MAO-B [43], a retrospective analysis in parkinsonian patients was performed. The long term (9 years) effect of treatment with Madopar alone (N=177) or in combination with Madopar+DEP (N= 564) revealed a significant increase in life expectancy in Madopar+DEP group regardless of the significant demographic differences between the two groups [46].

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 20.000-times higher dose is tolerated without any sign of toxic effect. This is truly an exceptional safety margin [3].

Conclusion

Since the first longevity study with the low enhancer doses of DEP (0.001 or 0.1 mg/kg) or BPAP (0.0001 or 0.05 mg/kg) revealed that enhancer treated rats lived longer than their saline-treated peers, the study demonstrated that the enhancer effect is responsible for longevity. The fact that 18-month-old rats, treated with 0.0001 mg/kg BPAP, were as good of performers in the shuttle box as three-month-old saline-treated rats, illustrated the special role of the enhancer regulation in improving the quality of mammalian life.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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