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

Chapter 8

The anti-aging effect of DEP and BPAP

We can define enhancer-regulations as: the existence of enhancer-sensitive neurons capable of changing their excitability in milliseconds and working on a higher activity level, due to natural or synthetic enhancer substances (Knoll 2005, 2016).

Chapters 3 and 4 showed that the catecholaminergic and serotonergic neurons were identified and analyzed in detail as the first models of life important enhancer-sensitive brain regulations.

Analyzing the molecular mechanism of (2*R*)-1-(1-benzofuran-2-yl)-*N*-propylpentane-2-amine (BPAP)'s specific and non-specific enhancer effects we clarified that the interaction with distinct sites on the vesicular monoamine-transporter-2 (VMAT-2) is the main mechanism of action of the enhancer substances. This finding elucidates BPAP's highly characteristic bi-modal, bell-shaped concentration-effect curves.

The discovery that natural enhancers maintain biological vigor during the developmental-phase of life, from weaning until sexual maturity, on a hyperactive level (Knoll and Miklya 1995); sexual hormones return the enhancer's activity back to its pre-weaning low level (Knoll, Miklya, Knoll and Dallo 2000); and due to post-developmental continuously progressing diminishment in the natural stimulation of the enhancer-sensitive brain regulations, mammals life is exactly limited to their technical life span (Knoll 2012).

As summarized in detail in Chapter 6, we presented unequivocal experimental evidence that as soon as sexual hormones terminate the developmental/uphill phase of life; the slow, continuous, progressively aging enhancer-sensitive brain-regulations begin and last until death. The amount of the natural enhancers show, over time, a downward tendency and the consequences are exactly measurable with the continuous, progressive decline of the appropriate brain functions.

We finally published in 2016 the first longevity study demonstrating that the enhancer effect of the DEP and BPAP are responsible for life extension, and using the enhancer-sensitive dopaminergic neuron as experimental model, we presented the first experimental evidence in a longevity study that since enhancer-sensitive neurons do not age we can significantly extend the life expectancy of mammals by maintaining them during their post-developmental phase of life, on a low daily dose of a synthetic enhancer substance (Knoll and Miklya 2016).

DEP's pharmacological spectrum is unique. It is worthwhile to briefly recall that prior to the discovery of the enhancer-sensitive brain regulations the peculiar mode of DEP's effect on the nigrostriatal dopaminergic neurons appeared to represent a hitherto unknown brain regulation. This view was supported by a successful biochemical analysis.

We found in the early 1980s that the striata of rats treated with 0.25 mg/kg DEP daily for three weeks released five times more dopamine (DA) in the resting state and seven times more DA in response to KCl stimulation than the striata removed from rats treated daily with 0.1 ml/100 g saline. The striata were removed 24 hours after the last injection of saline or DEP, respectively. DEP increased the rate of utilization of DA in the striatum of DEP-treated rats. The increase in the turnover rate of DA in the striatum was due to the enhancement of the fractional rate constant of DA efflux and the significant increase in the DA content. We soon realized that the facilitation of striatal dopaminergic neurotransmission by long term treatment is highly specific. With regard to noradrenaline a significant decrease in the turnover rate and unchanged level of this amine in the brain stem was found, and no change in the turnover rate of serotonin (SE)-treated daily with 0.25 mg/kg DEP for two weeks was detected (Zsilla and Knoll 1982; Zsilla, Szekely and Knoll 1986).

Now we know that DEP is a PEA-derived almost specific synthetic CAE substance, which being the only one free of the catecholamine-releasing property of PEA, its natural parent compound, made it possible to discover the operation of the enhancer-sensitive regulations in the mammalian brain (Knoll 1998).

The discovery of the enhancer-sensitive brain regulations and the development of DEP and BPAP, the synthetic enhancers, allowed a new approach to better understand the essence of brain aging and elaborate a previously unimaginable, simple and safe method to prevent the manifestation of the regressive effect of brain aging. To characterize the essence of brain aging we analyzed the aging-related decline of two dopaminergic functions: sexual activity and learning ability.

The Aging-related Decline of Sexual Activity

It is fascinating to compare the astonishing similarity in human and rat males in the aging-related decline of the mesencephalic dopaminergic system and realize the same functional consequences, the progressing weakening and final extinction of the ejaculatory activity during their postdevelopmental phase of life .

Sexual activity in the human male is known to be influenced by a number of factors, such as good health, stable marriage, satisfactory sexual partner(s) and adequate financial and social status. But even in the males who meet all the requirements for retention and maintenance of sexual functioning, there is an age-related decrease in sexual vigor.

In the Baltimore Longitudinal Study of Aging, coital activity was studied as function of age. They interviewed 628 members of the Washington-Baltimore area, varying from 20-95 years of age, white, married, urban residents in good health. According to this study the median coital activity was highest, ***2.1*** events/week, between ages of 30-34, and decreased progressively with increasing age, sinking to ***0.2***/week in the age-group 65-69.

It is common knowledge that individual variation in sexual vigor is enormous. In this study the mean frequency of total sexual activity in 159 males was found to be 520 sexual events/5 years in the age-group 20-39, including young males performing below 100 sexual events/5 years and those with frequencies of total sexual activity over 1000 sexual events/5 years. In the age-group 65-79, the mean frequency of total sexual activity decreased to 75 sexual events/5 years, but even in this group subjects producing 400-700 sexual events/5 years were registered (Martin 1977).

In a number of longitudinal studies performed on male rats we observed that the age-related decline of coital activity in male rats and the striking individual differences in sexual performance in different age cohorts are essentially the same as in human males (Knoll 1988, 1989, 1990; Knoll, Dallo and Yen 1989; Martin 1977). Because of brain aging, even the most sexually high performing males may lose their potency to ejaculate if they live long enough. In our studies on male CFY rats, we followed the sexual performance of the animals once a week from sexual maturity until death. We measured three patterns: mounting, intromission and ejaculation. We found that in response to brain aging even the best performing individuals lost their potency to ejaculate no later than the completion of their second year of age (Knoll 1990). The results of our first longevity study clearly proved in retrospect that the age-related decline

of the sexual performance of male rats signals the decay of the enhancer regulation in the dopaminergic neurons over time (Knoll 1988, 1989; Knoll, Yen and Miklya 1994).

In this series of experiments, we selected 132 aged, *2-year-old* male rats and measured in four consecutive weekly mating tests their sexual performance: mounting, intromission and ejaculation. Due to aging, the ability to ejaculate ceased in 2-year-old CFY rats. We classified the rats according to their sexual performance in the testing period as non-copulators (no sign of sexual activity), mounting rats (displayed mounting only) and sluggish rats (displayed mounting and intromission). Of the 132 rats 46 were found to be non-copulators (Group 1), 42 displayed mounting only (Group 2) and 44 rats proved to be sluggish (Group 3). After the selection period, we started to treat half of the rats with saline (1 ml/kg) and half with DEP (0.25 mg/kg) three times a week, until they died. We tested their sexual performance once a week. The dying out of the 66 saline-treated rats showed that lifespan was proportional to their sexual performance. As sexual performance is directly proportional to the functional state of the enhancer regulation in the dopaminergic neurons, we assume that rats die when the age-related decline in mesencephalic enhancer regulation reached a critical threshold. With regard to sexual performance: Group 1 < Group 2 < Group 3, thus, rats belonging to Group 1 are the closest to exceeding the critical threshold resulting in natural death and die out first; rats in Group 2 live longer; and rats in Group 3 live the longest.

The age-related decline in mesencephalic enhancer regulation during the post-developmental phase of life in male rats can be further recognized by comparing the individual variation in sexual performance of 3-6-month-old male rats with the performance of their 2-year-old peers. Whereas 52.49% of 3-6-month-old male rats displayed ejaculations during the four consecutive mating tests, only 5.80% of 12-18-month-old males ejaculated and none of the 24-month-old males were endowed with this faculty any longer (Knoll 2005).

Moreover, the age-related change in the percentage of animals belonging to the “non-copulator” group clearly proved that enhancer regulation in the dopaminergic neurons is in continuous decline during the post-developmental phase of life. Only 5.51% of the 3-6-month-old males were sexually inactive, but 19.56% of the 12-18-month-old rats and 34.84% of the 24-month-old rats belonged to this group.

Due to the striking similarities between human and rat males in the age-related decline of their sexual activity it is hard to deny that the decay of the dopaminergic machinery over time plays the key role on the final loss of the ability to ejaculate, from which there is no escape. We

demonstrated with a series of experiments that the treatment of male rats with DEP significantly enhanced their sexual activity and with the preventive administration of a small dose of DEP the loss of the ability to ejaculate was substantially shifted in time (Knoll 1988, 1989, 1990, 1993; Knol, Dallo and Yen 1989; Knoll, Yen and Miklya 1994; Martin 1977).

In Table 8.1, a brief summary of the results of our first longevity study shows that the anti-aging effect of DEP was decisive even in a series of experiments performed on two-year-old rats which had already lost their ability to ejaculate.

Table 8.1. Illustration of the antiaging effect of DEP-treatment on 2-year-old rats. Data taken from the first longevity study (Knoll, Yen and Miklya 1994).

Classification of the groups according to sexual performance before treatment	**Number of animals**	**Total number of mountings (M), intromissions (I) and ejaculations (E) of the groups during treatment**		
		M	**I**	**E**
		Saline-treated rats		
Non-copulators	23	37	0	0
Mounting rats	21	425	54	0
Sluggish rats	22	477	231	0
		DEP-treated rats		
Non-copulators	23	997	544	190
Mounting rats	21	1129	662	172
Sluggish rats	22	1696	1257	481

A second example demonstrates on young male CFY rats the DA-dependency of sexual performance and the significant anti-aging effect of DEP-treatment. We selected 90 males possessing full-scale sexual activity. Half of the population was treated with saline (1 ml/kg), the other half with DEP (0.25 mg/kg), three times a week, from the 25th week of age. The rats' sexual performance was tested once a week. In this study, the loss in the ability to ejaculate was selected as the age-related end stage. Saline-treated rats reached this stage at an average of ***112±9*** weeks. In contrast, DEP-treated rats reached it at an average of ***150±12*** weeks (P<0.001) (Knoll 1993). As sexual performance is a dopaminergic function, it became obvious that the enhanced activity of the mesencephalic dopaminergic neurons was responsible for the significantly retarded loss of the ability to ejaculate in the DEP-treated group.

The Dopamine-dependent Age-related Decline of Learning Ability

In a modified version of the shuttle box, originally described by Bovet, Bovet-Nitti and Oliverio (1966), the acquisition of a two-way conditioned avoidance reflex (CAR) was analyzed over five consecutive days. The rat was put 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 punished with a foot shock (1mA), the unconditioned stimulus (US) and 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. At each learning session, the number of CARs, EFs and inter-signal reactions (IRs) were automatically counted and evaluated by multi-way ANOVA.

The dopaminergic machinery is the most rapidly aging neuronal system in our brain. The dopamine content of the human caudate nucleus decreases steeply, at a rate of 13% per decade over age 45. We know that symptoms of Parkinson's disease (PD) appear if the DA content of the caudate sinks below 30% of the normal level. Experimental and clinical experiences show that daily DEP-dosages keep the brain engine 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 in healthy humans against the age-related decrease in striatal DA, 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 in lifespan, which is now estimated to be around 115 years (Knoll 1992).

Due to aging of the dopaminergic neurons, saline-treated 3-month-old rats are significantly better learners than their saline-treated 1-year-old peers (Figure 8.1). Since synthetic enhancers keep the dopaminergic neurons working on a higher activity level, rats treated with 0.1 mg/kg DEP (Figure 8.2), or 0.0001 mg/kg BPAP (Figure 8.3), showed no sign of aging-related decay in the learning ability

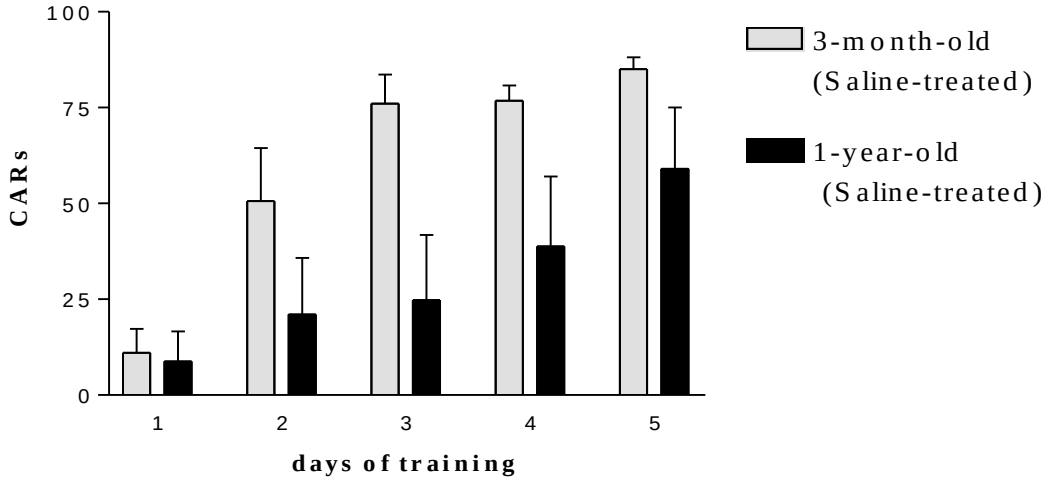

Figure 8.1. Experimental evidence that 3-month-old rats are significantly better learners than their 1-year-old peers ($P<0.001$). Significance in the performance between the groups was evaluated by multi-factor analysis of variance (ANOVA). Rats were trained in the shuttle box with 100 trials per day. Conditioned avoidance responses (CARs).

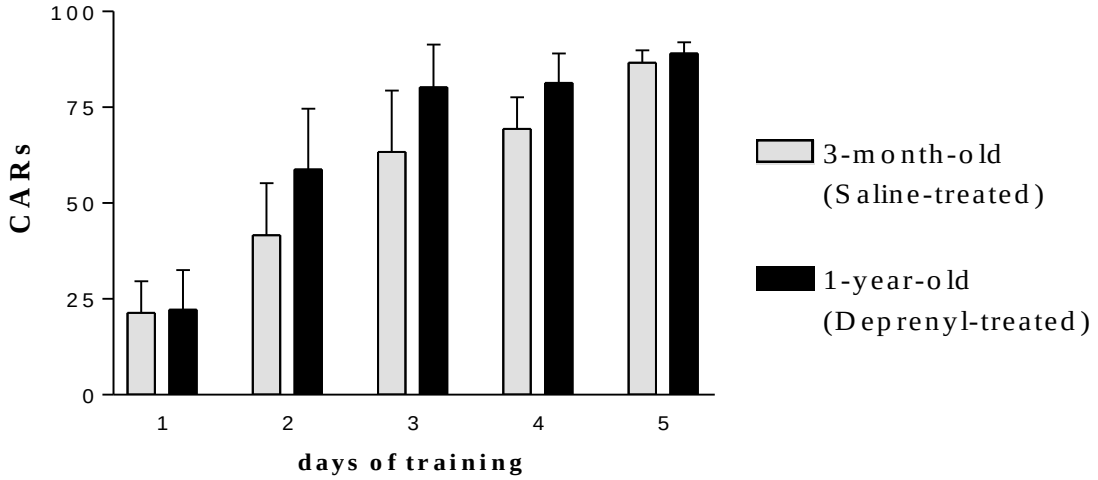

Figure 8.2. Experimental evidence shows that in rats treated with 0.1 mg/kg DEP there is no sign of aging-related decay in the learning ability. Rats were trained in the shuttle box with 100 trials per day. Significance in the performance between the groups was evaluated by multi-factor analysis of variance (ANOVA). There was no significant difference in the acquisition of conditioned avoidance responses (CARs) between the 3-month-old rats treated with saline and 1-year-old rats treated with DEP.

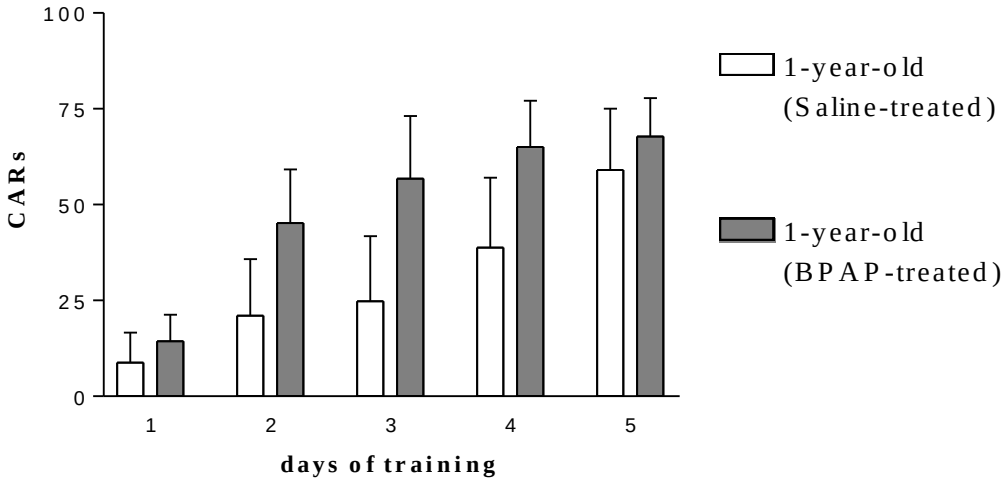

Figure 8.3. The anti-aging effect of 0.0001 mg/kg BPAP. 1-year-old rats were treated for 10 months subcutaneously, 3-times a week, with 0.0001 mg/kg BPAP. Their performance was compared to saline-treated rats. Rats were trained in the shuttle box with 100 trials per day. Significance in the performance between the groups was evaluated by multi-factor analysis of variance (ANOVA). BPAP treated rats performed significantly better than their saline-treated peers ($p<0.05$)

The Enhancer-sensitive Neurons Do Not Age

We realized in preliminary studies that in contrast to the rapidly aging natural enhancers, the enhancer-sensitive neurons do not age, thus the synthetic enhancers are capable to substitute the lost natural enhancers, thus to prevent the regressive effects of brain aging. To present unequivocal experimental evidence that enhance-sensitive neurons remain sensitive toward synthetic enhancers, 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 (Knoll and Miklya 2016).

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. Differences were considered significant at $p<0.05$.

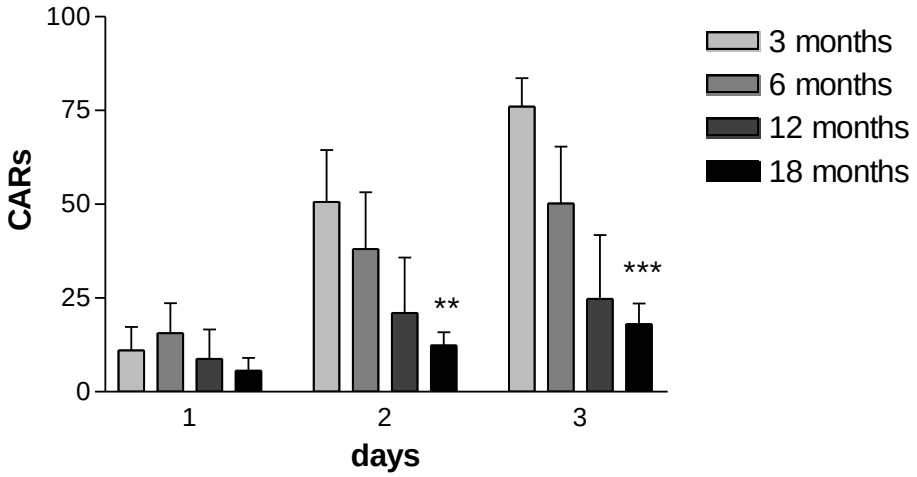

Figure 8.4. The age-related physiological decline in the learning ability of 3-, 6-, 12- and 18-month-old saline-treated rats. CARs-conditioned avoidance responses. Two-way ANOVA followed by Bonferroni post-hoc test: CAR age $F(3/48)=5.961$ $^{**}p<0.01$; days $F(2/48)=8.746$

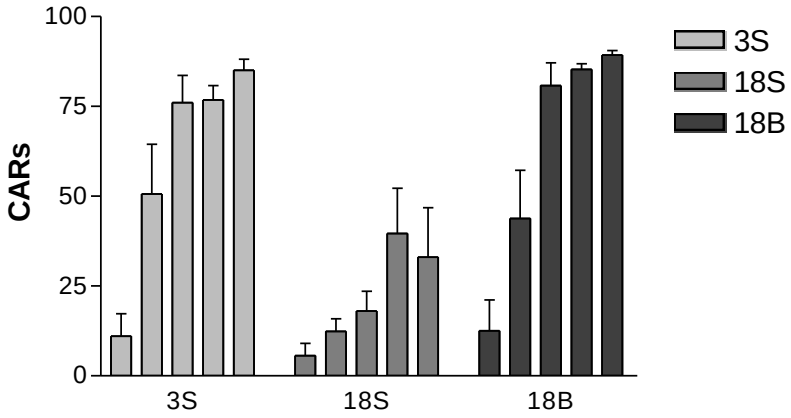

Figure 8.5.
3S: The full capacity of 3-month-old saline-treated rats in their ability to fix a condition avoidance response in the shuttle box during training for 5 consecutive days.
18S: The aging-related serious decline in the ability of saline-treated 18-month-old rats in building a condition avoidance 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; $F(8/55)=2.284$ $^{*}p<0.05$.

Figure 8.4 shows the conditioned avoidance responses in groups of 5 saline-treated rats selected from the longevity study. The young, 3-month-old rats showed their normal performance; the 6-month-old group of rats already showed a considerable decline in their

learning performance. The 12-month-old rats gave evidence of further loss in their ability to build CARs, and 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).

This longevity study was the first demonstration that lifelong treatment with 0.0001 mg/kg BPAP, the peak dose with the specific enhancer effect, completely prevented aging of learning ability. We used the learning test as a highly sensitive model to measure the aging-related decay of the dopaminergic neurons. The BPAP-treated 18-month-old rats performed in the shuttle box like the saline-treated 3-month-old rats. This finding is an unprecedented, convincing proof that the enhancer-sensitive dopaminergic neurons do not age. Thus, the development of the first synthetic enhancer substances guides the way to prevent the regressive effects of brain aging.

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