
3 Enhancer Regulation: A Neurochemical Approach to the Innate and Acquired Drives

3.1

Mesencephalic Enhancer Regulation: Natural and Synthetic Mesencephalic Enhancer Substances

3.1.1

Definition of Enhancer Regulation: β -Phenylethylamine (PEA) and Tryptamine, Endogenous Enhancer Substances

We can define enhancer regulation as: the existence of enhancer-sensitive neurons capable of changing their excitability in a split second and working on a higher activity level, due to natural enhancer substances. Of the agents with such effect, for the time being, only β -phenylethylamine (PEA) and tryptamine have been experimentally analyzed (Knoll 2001, 2003).

Though enhancer sensitive neurons exist also outside the mesencephalon, we used the term “mesencephalic enhancer regulation” to emphasize the key importance of the dopaminergic neurons, the most rapidly aging neurons of the brain, primarily responsible for the progressive age related decline of behavioral performances.

The catecholaminergic and serotonergic neurons in the mesencephalon are excellent models to study the enhancer regulation since their physiological function is to supply the brain continuously with the proper amounts of monoamines that influence – activate or inhibit – billions of neurons. The significant enhancement of the nerve-stimulation-induced release of [^3H]-nor-epinephrine, [^3H]-dopamine, and [^3H]-serotonin from the isolated brain stem of the rat in the presence of PEA (Fig. 3.1) or tryptamine (Fig. 3.2) is shown to illustrate the response of enhancer-sensitive neurons to endogenous enhancer substances.

From a freshly isolated brain stem of a properly pretreated rat a low amount of the labeled transmitters is released for a couple of hours (see Knoll and Miklya 1995, for methodology). Neurons respond to stimulation in an “all or none” manner. The calculated average amount of each of the transmitters released from the non-stimulated brain stem is the product of the spontaneous firing of the most excitable, most responsive group of neurons of the surviving population with large individual variation in excitability. The overwhelming

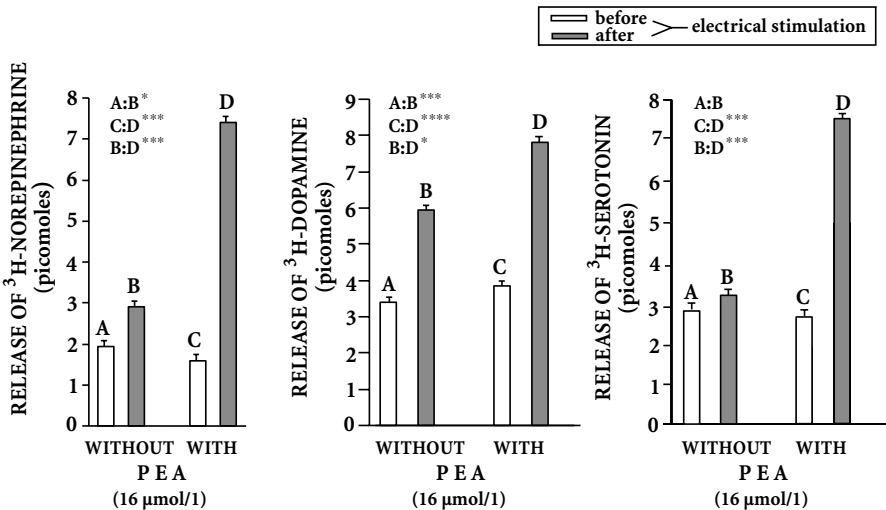


Fig. 3.1. Significant enhancement of the nerve-stimulation-induced release of [³H]-norepinephrine, [³H]-dopamine, and [³H]-serotonin, respectively, from the isolated brain stem of the rat in the presence of β -phenylethylamine (PEA) ($n = 8$). Each graph bar represents the amount of the labeled transmitter in picomoles released in a 3-min collection period. See Knoll et al. (1996c) for methodology. Vertical lines above the graph bars show the SEM (standard error of the mean). Paired Student's t -test was used for statistical analysis. * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.001$

majority of the neurons remain silent. Electrical stimulation excites a further group of neurons as shown by the significant increase of the outflow of transmitters. Natural enhancer substances increase specifically the excitability of the enhancer-sensitive neurons. Accordingly, Figs. 3.1 and 3.2 demonstrate that in the presence of PEA or tryptamine the amount of transmitters released to electrical stimulation increased dramatically.

The data in Figs. 3.1 and 3.2 show a remarkable quantitative difference between PEA and tryptamine in their effectiveness on serotonergic neurons. A lower concentration of tryptamine ($1.3 \mu\text{mol/l}$) proved to be much more potent in enhancing the stimulation-evoked release of serotonin than a much higher concentration of PEA ($16 \mu\text{mol/l}$). This indicates that, on a molecular level, the enhancer regulation in the catecholaminergic and serotonergic neurons are not identical.

Enhancer regulation in the brain heralds a new line of research. It brings a different perspective to brain-organized, goal-oriented behavior since it seems to represent the device in the mammalian brain that operates as the *vis vitalis*.

PEA and tryptamine, the first examples of physiological enhancer substances, represent just the peak of an iceberg. The development of a tryptamine-derived synthetic enhancer substance that increased the performance of cul-

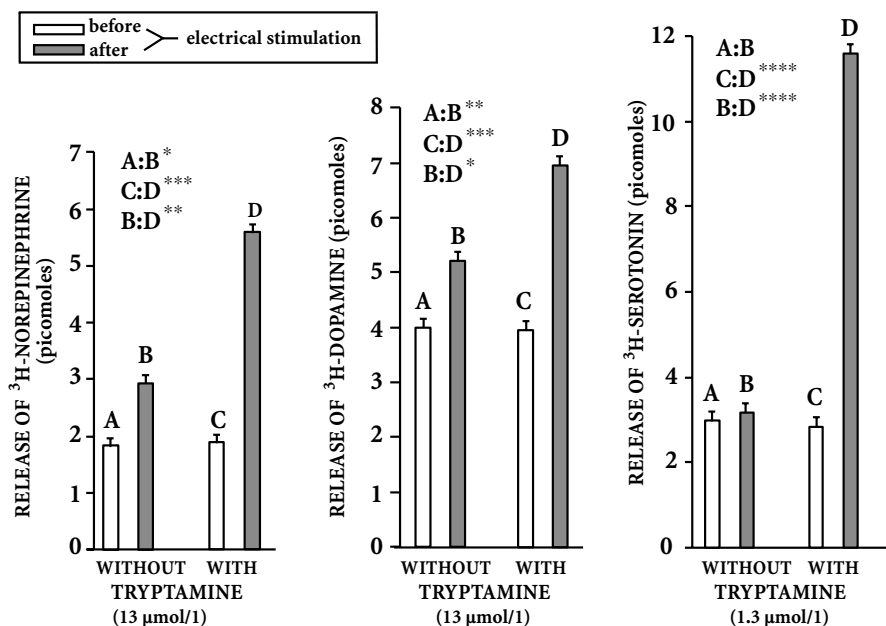


Fig.3.2. Significant enhancement of the nerve-stimulation-induced release of [^3H]-norepinephrine, [^3H]-dopamine, and [^3H]-serotonin, respectively, from the isolated brain stem of the rat in the presence of tryptamine. ($n = 8$). Each graph bar represents the amount of the labeled transmitter in picomoles released in a 3-min collection period. See Knoll et al. (1996c) for methodology. Vertical lines above the graph bars show the SEM. Paired Student's t -test. * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.001$

tured hippocampal neurons with a peak effect at 10^{-14} M concentration (see Fig. 5 in Knoll et al. 1999) foreshadows the existence of much more potent physiological enhancer substances in the brain than PEA and tryptamine and incites research in this direction.

3.1.2

The Role of (–)-Deprenyl in the Recognition of the Enhancer Regulation in the Mesencephalic Neurons

A thorough, 30-year analysis of the mechanism of action of (–)-deprenyl, a drug presently registered world-wide for the treatment of Parkinson's disease, ultimately resulted in the recognition of enhancer regulation in the mesencephalic catecholaminergic neurons (see Knoll 1998, for review). The history how this crucially important physiological mechanism remained undetected for decades gives a good example of concealed traps in research.

We developed (–)-deprenyl in the early 1960s (see Knoll 1983, for a review of the first two decades of its history). When we started to develop (–)-deprenyl,

MAO inhibitors were at the center of interest. Both as experimental tools and as therapeutic agents MAO inhibitors had an important influence on the development of the widely accepted hypothesis: that 1. depression is associated with diminished monoaminergic tone in the brain, and 2. depressed patients treated with antidepressants become elated because of enhanced biological activity of monoamine transmitters in the central nervous system.

The discovery of the mood-elevating effect of MAO inhibitors was a classic example of serendipity in drug research. In 1951, isoniazid and its isopropyl derivative, iproniazid, were successfully introduced for the treatment of tuberculosis. In contrast to isoniazid, iproniazid was found to produce undesirable stimulation in some patients. In 1952, Zeller and his co-workers demonstrated that iproniazid was capable of inhibiting MAO, whereas isoniazid was ineffective (Zeller and Barsky 1952; Zeller et al. 1952). In 1956, Crane analyzed the psychiatric side-effects of iproniazid and came to the conclusion that it might be beneficial in the treatment of depression (Crane 1956). In 1957 Kline introduced it as a "psychic energizer" (Kline 1958). At the same time Kuhn discovered the antidepressant effect of imipramine (Kuhn 1957). This opened the way to the most powerful antidepressant therapy to date.

At the beginning there was a keen interest in the MAO inhibitors, of which a substantial number were developed and introduced into clinical practice, but because of serious side-effects there was a rapid turnover in the introduction and withdrawal of these drugs. In 1963, a calamitous number of clinical reports, demonstrating the occurrence of dangerous hypertensive attacks in patients treated with MAO inhibitors were published. Blackwell suggested that the hypertensive crises are associated with the ingestion of high amounts of tyramine in cheese, the metabolism of which is inhibited by the MAO inhibitors ("cheese effect") (Blackwell 1963). This conclusion was correct. Cheese and many other foods containing tyramine were found to be able to provoke hypertensive episodes in patients treated with MAO inhibitors. The "cheese effect" restricted the clinical use of this group of drugs.

Deprenyl (we used the racemic compound under the code name E-250 in the first series of experiments) proved to be a compound with a peculiar pharmacological spectrum. We described it in our first paper as a new spectrum psychic energizer (Knoll et al. 1965). I selected this compound for further development because I was fascinated by the finding that in contrast to MAO inhibitors, which potentiated the blood pressure increasing effect of amphetamine, a releaser of norepinephrine from their stores in the noradrenergic nerve terminals, E-250 *inhibited* it (see Fig. 1 in Knoll et al. 1965). Based on this observation we analyzed this peculiar behavior in more detail. As I expected, the studies revealed that *deprenyl, 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 performed on cats and on the isolated vas deferens of rats. The hope was expressed in this paper that this

peculiar tyramine-inhibiting property of a potent MAO inhibitor might be of special therapeutic value (Knoll et al. 1968).

In the same year that the description of the unique behavior of (–)-deprenyl was published, Johnston described a substance, later named clorgyline, that came into world-wide use as an experimental tool in MAO research. Johnston realized that clorgyline preferentially inhibits the deamination of serotonin. He proposed the existence of two forms of MAO, “type A” and “type B,” the former being selectively inhibited by clorgyline and the latter relatively insensitive to it. Johnston’s nomenclature has become widely accepted and is still in use (Johnston 1968).

For further studies a selective inhibitor of MAO-B was needed. We were lucky to discover in 1970 that (–)-deprenyl was the missing, highly selective inhibitor of MAO-B (Knoll and Magyar 1972). The compound was used thereafter as the specific experimental tool to analyze MAO-B. Our first paper that described this novel property became a citation classic 10 years later (Knoll J, This Week’s Citation Classic, January 15, 1982). For several years the selective MAO-B inhibitory effect was at the center of our interest. It delayed the discovery of the drug’s enhancer effect. It was the MAO inhibitory effect of the compound that led to the first clinical application of (–)-deprenyl.

In light of the serious side effects of levodopa in Parkinson’s disease, Birkmayer and Hornykiewicz tried to achieve a levodopa-sparing effect by the concurrent administration of levodopa with an MAO inhibitor. As such combinations frequently elicited hypertensive attacks, they were soon compelled to terminate this line of clinical research (Birkmayer and Hornykiewicz 1962).

We had already shown in animal experiments that (–)-deprenyl is a unique MAO inhibitor which does not potentiate the catecholamine-releasing effect of indirectly acting amines, but instead inhibits it. We proposed to use it as an MAO inhibitor free of the cheese effect (Knoll et al. 1968). The validity of this proposal was shown in man by Sandler and his co-workers (Elsworth et al. 1978; Sandler et al. 1978). Considering the peculiar pharmacological profile of (–)-deprenyl, Birkmayer et al. (1977) dared to combine this MAO inhibitor with levodopa in Parkinson’s disease. This trial was successful. The levodopa-sparing effect was achieved in parkinsonians without signs of significant hypertensive reactions. This study initiated the world-wide use of (–)-deprenyl in Parkinson’s disease.

Today the most evaluated effect of the drug is its ability to slow the rate of the functional deterioration of the nigrostriatal dopaminergic neurons in patients with early, untreated Parkinson’s disease, and thus to slow the progress of the disease. The indication for using (–)-deprenyl in *de novo* parkinsonians was established in the DATATOP study in the USA (Tetrud and Langston 1989; Parkinson Study Group 1989, 1993) and was corroborated in important multicenter studies (Allain et al. 1991; Myttila et al. 1992; Larsen et al. 1999).

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 (–)-deprenyl. As a matter of fact, in the DATATOP multicenter study of the Parkinson Study Group a segment of this continuum, the time elapsing from diagnosis of Parkinson's disease until levodopa was needed, was properly measured in untreated patients with Parkinson's disease and the effect of (–)-deprenyl versus placebo was compared (Parkinson Study Group 1989). Tetrud and Langston (1989) were the first to publish the finding that (–)-deprenyl 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 (–)-deprenyl group. This was clear proof that (–)-deprenyl, which enhances the activity of the surviving dopaminergic neurons, kept these neurons on a higher activity level for a longer duration of time.

The design of the DATATOP study was unintentionally the same that we had used in our rat experiments with (–)-deprenyl since 1980. We tested the sexual activity of male rats as a quantitatively measurable, rapidly aging dopaminergic function, and compared the effect of (–)-deprenyl versus saline treatment on the age-related decline of copulatory activity in rats. We demonstrated that (–)-deprenyl treatment significantly slowed the age-related decay of sexual performance (Knoll 1982) and later went on to show that this effect of (–)-deprenyl was unrelated to the inhibition of MAO-B. We performed a structure–activity relationship study aiming to select a derivative of (–)-deprenyl that is free of any MAO-B inhibitory property (Knoll et al. 1992a). In (–)-deprenyl the propargyl group binds covalently to the flavin group of MAO-B, and this leads to the irreversible inhibition of the enzyme activity. (–)-PPAP, the new (–)-deprenyl analogue selected, 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 (–)-deprenyl, but did not change the activity of MAO-B. One can follow the progress in clarifying the mechanism of action of (–)-deprenyl responsible for enhanced dopaminergic activity in a series of reviews (Knoll 1978, 1983, 1987, 1992, 1995, 1998, 2001, 2003).

By now it is clear that if we select a quantitatively measurable dopaminergic function and determine its age-related decline by fixing an exact end, a shift of this end stage in time in (–)-deprenyl-treated rats shows the dopaminergic activity enhancer effect of the drug. For example, male rats ultimately lose their ability to ejaculate due to the physiological aging of the striatal dopaminergic system. We found that saline-treated rats reached this stage at the age of 112 ± 9 weeks, whereas their (–)-deprenyl-treated peers lost the ability to ejaculate only at the age of 150 ± 12 weeks ($P < 0.001$) (Knoll 1992).

The design of the DATATOP study was essentially the same. The authors knew that after having diagnosed Parkinson's disease the next step would be

the need for levodopa, and they measured the (–)-deprenyl-induced delay in reaching this stage.

The authors of the DATATOP study expected (–)-deprenyl 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 Parkinson's disease. In accord with their working hypothesis they expected that (–)-deprenyl, 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 Parkinson's disease and measured the delay of the onset of disability necessitating levodopa therapy.

In the first part of the trial 401 subjects were assigned to α -tocopherol or placebo and 399 subjects were assigned to (–)-deprenyl, alone or with α -tocopherol. Only 97 subjects who received (–)-deprenyl 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 (–)-deprenyl. The risk of reaching the end of the trial was reduced by 57% for the subject who received (–)-deprenyl, 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 (–)-deprenyl, but not α -tocopherol, delayed the onset of disability associated with early, otherwise untreated Parkinson's disease. But as time passed, the DATATOP study also revealed that (–)-deprenyl did not reduce the occurrence of subsequent levodopa-associated adverse effects in the patients (Parkinson Study Group 1996). A comparison of the enhancer effect of α -tocopherol with that of (–)-deprenyl showed that α -tocopherol did not change the impulse-evoked release of norepinephrine, dopamine and serotonin in the brain, thus it is devoid of an enhancer effect (Miklya et al. 2003a).

Although Tetrad and Langston and other authors of the DATATOP study were not aware of the dopaminergic activity enhancer effect of (–)-deprenyl, their trial was the first to give convincing evidence that (–)-deprenyl, in harmony with our findings in rats, keeps the nigrostriatal dopaminergic neurons on a higher activity level in humans. In addition, this effect of (–)-deprenyl had already been detected in a selected human population with the lowest striatal dopaminergic activity. The highly significant effect of (–)-deprenyl and the ineffectiveness of α -tocopherol during the first years of the DATATOP study were clear proof that (–)-deprenyl acted by enhancing the activity of the nigrostriatal dopaminergic neurons. The patients selected for the study with early, untreated Parkinson's disease were ideal for demonstrating this effect. The subjects still had a sufficient number of dopaminergic neurons whose

activity could be enhanced by (–)-deprenyl; thus, the need for levodopa therapy was delayed. α -Tocopherol, devoid of a dopaminergic activity enhancer effect, remained ineffective. As Parkinson's disease is incurable, drug effects are necessarily transient in nature. It is obvious that parallel with further decay of the striatal dopaminergic system, the responsiveness of the patients toward (–)-deprenyl decreased with the passing of time (Parkinson Study Group 1996).

With the development of (–)-1-phenyl-2-propylaminopentane, (–)-PPAP, the (–)-deprenyl analogue free of the MAO-B inhibitory potency, we already furnished direct evidence that the enhanced dopaminergic activity following administration of (–)-deprenyl was unrelated to the inhibition of MAO-B. Because (–)-PPAP, like (–)-deprenyl, inhibited the uptake of tyramine in isolated smooth muscle tests, we first assumed that the drug-induced enhanced dopaminergic activity was due to an uptake inhibitory effect. Further studies revealed that this interpretation was false.

The availability of HPLC to measure catecholamines and serotonin in physiological quantities allowed a new approach. The thorough analysis of the dose-dependent effect of (–)-deprenyl on the release of catecholamines and serotonin from isolated, discrete, rat brain regions (dopamine from the striatum, substantia nigra and tuberculum olfactorium; norepinephrine from the locus coeruleus; and serotonin from the raphe) pointed to enhancer regulation in the mesencephalic neurons. We treated rats with 0.01, 0.025, 0.05, 0.1 and 0.25 mg/kg (–)-deprenyl, respectively, once daily for 21 days, isolated the discrete rat brain regions 24 h after the last injection and measured the biogenic amines released during a 20-min period from the freshly isolated tissue samples. The amount of dopamine released from the substantia nigra and tuberculum olfactorium clarified that the dopaminergic neurons worked on a significantly higher activity level even in rats treated with the lowest dose of (–)-deprenyl, 0.01 mg/kg. As this small dose of (–)-deprenyl leaves MAO-B activity and the uptake of amines practically unchanged, this study was the first unequivocal demonstration of the operation of a hitherto unknown enhancer mechanism in dopaminergic neurons stimulated by (–)-deprenyl in very low doses (Knoll and Miklya 1994).

Further studies clarified the operation of mesencephalic enhancer regulation (Knoll and Miklya 1995; Knoll et al. 1996a,b,c). We realized that PEA, the parent compound of (–)-deprenyl, is primarily an endogenous mesencephalic enhancer substance. Since PEA, in higher concentrations, is a highly effective releaser of catecholamines from their intraneuronal stores, this effect covered up completely the enhancer effect of this endogenous amine, which was classified as the prototype of the indirectly acting sympathomimetics.

Amphetamine and methamphetamine, PEA derivatives with a long lasting effect, share with their parent compound the releasing property. (–)-Deprenyl was the first PEA/methamphetamine derivative that *maintained the enhancer*

effect of its parent compounds but *lost completely the releasing property*. This peculiar change in the pharmacological spectrum of this PEA derivative ultimately enabled the discovery of the enhancer regulation in the mesencephalic neurons, since the enhancer effect of (–)-deprenyl was not covered up by the release of catecholamines from their intraneuronal stores.

In the light of our present knowledge clinicians were mistaken from the very beginning who used (–)-deprenyl in the belief that the therapeutic benefits observed in patients treated with this drug were due to the selective inhibition of MAO-B in the brain. The overwhelming majority of the clinical benefits are due to the enhancer effect of (–)-deprenyl (see Knoll 1998, for review).

3.1.3

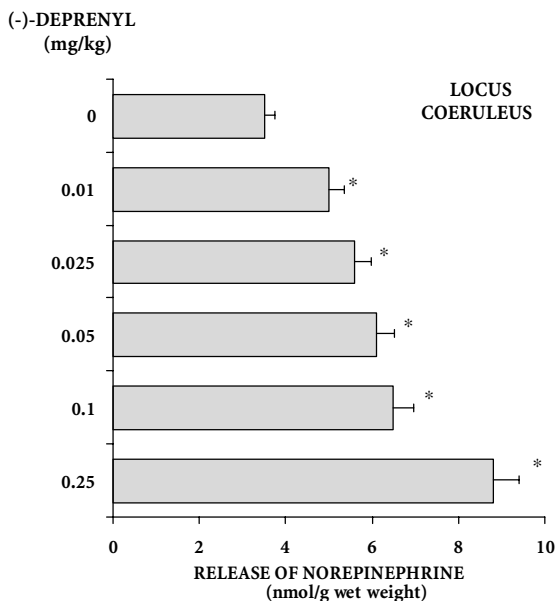
(–)-Deprenyl (Selegiline) and *R*-(–)-1-(benzofuran-2-yl)-2-propylaminopentane [(–)-BPAP], Prototypes of Synthetic Mesencephalic Enhancer Substances

3.1.3.1

(–)-Deprenyl, the PEA-Derived Enhancer Substance

(–)-Deprenyl (Selegiline), developed in the early 1960s as a new spectrum psychostimulant and potent MAO inhibitor, later proved to be, as the first selective inhibitor of MAO-B, indispensable for investigating the nature and function of B-type MAO. Hundreds of clinical studies with the drug were designed thereafter in the firm belief that selective blockade of MAO-B was responsible

Fig.3.3. Significant enhancement of norepinephrine release from the locus coeruleus of rats isolated 30 min after the subcutaneous administration of a single dose of (–)-deprenyl. The amount of norepinephrine released from the tissue within 20 min following the administration of different doses of (–)-deprenyl was measured according to Knoll and Miklya (1995). *Horizontal lines to the right of the graph bars show the SEM. Paired Student's *t*-test. **P* < 0.01*



for all the effects that followed (–)-deprenyl medication. Realizing, however, that PEA is an endogenous mesencephalic enhancer substance *and* a releaser of catecholamines, while (–)-deprenyl is a PEA-derived synthetic mesencephalic enhancer substance devoid of any catecholamine-releasing property, it became clear that the enhancer effect of (–)-deprenyl was responsible for the majority of the beneficial effects of the drug described in various experimental and clinical studies (see Knoll 1998, 2001, for review).

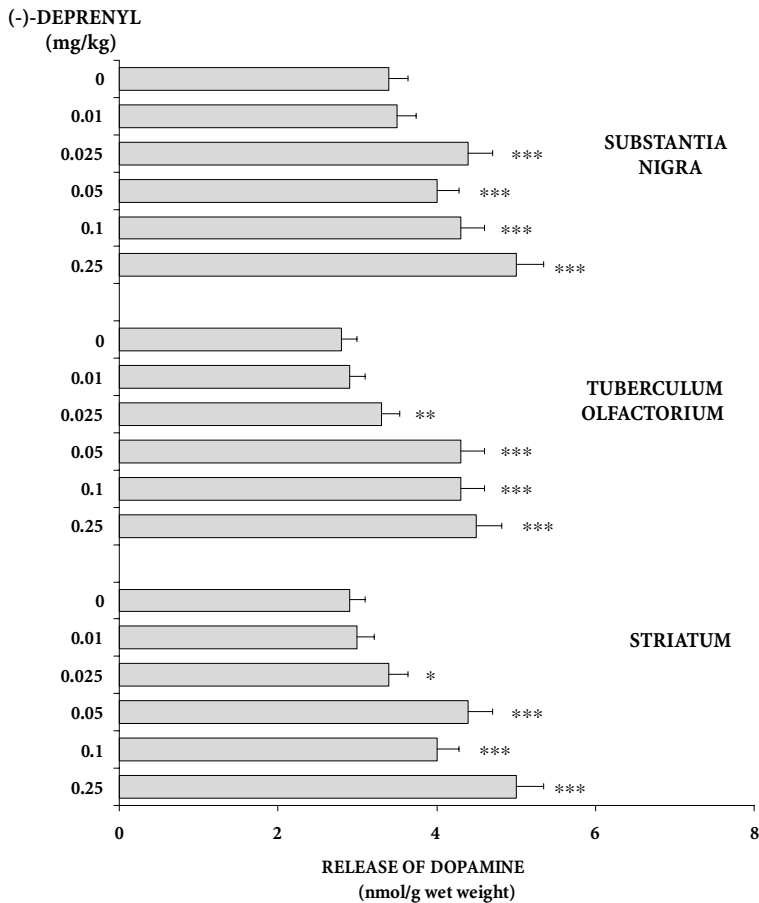


Fig.3.4. Significant enhancement of dopamine release from the substantia nigra, tuberculum olfactorium, and striatum of rats, respectively, isolated 30 min after the subcutaneous administration of a single dose of (–)-deprenyl. The amount of dopamine released from the tissue within 20 min following the administration of different doses of (–)-deprenyl was measured according to Knoll and Miklya (1995). *Horizontal lines to the right of the graph bars show the SEM. Paired Student's *t*-test. **P* < 0.05, ***P* < 0.02, ****P* < 0.01*

PEA, rapidly metabolized by MAO, is short acting and its enhancer effect can be detected in *in vitro* experiments only (see Fig. 3.1). Since (–)-deprenyl is not rapidly metabolized, its effect can be measured quantitatively *in vivo*. The subcutaneous administration of (–)-deprenyl enhanced the activity of the catecholaminergic neurons in a dose-dependent manner. This effect is shown on noradrenergic neurons (Fig. 3.3) and on dopaminergic neurons (Fig. 3.4). (–)-Deprenyl treatment, however, did not enhance the activity of serotonergic neurons (Fig. 3.5). (–)-Deprenyl is a PEA-derived enhancer substance and its *in vivo* ineffectiveness on serotonergic neurons is in accord with the finding that PEA was much less potent than tryptamine in enhancing the activity of the serotonergic neurons in the *in vitro* experiments, too (cf. Fig. 3.1 with Fig. 3.2).

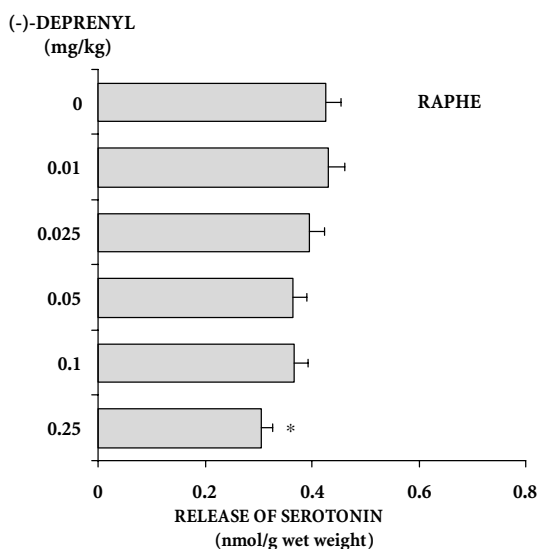


Fig. 3.5. Lack of enhancement of serotonin release from the raphe of rats isolated 30 min after the subcutaneous administration of a single dose of (–)-deprenyl. The amount of serotonin released from the tissue within 20 min following the administration of different doses of (–)-deprenyl was measured according to Knoll and Miklyá (1995). *Horizontal lines to the right of the graph bars show the SEM.* Paired Student's *t*-test was used for statistical analysis. None of the applied doses of (–)-deprenyl enhanced the release of serotonin significantly, the highest dose even decreased the release significantly. * $P < 0.05$

Since (–)-deprenyl is a highly potent and selective inhibitor of MAO-B, we performed a structure-activity relationship study to develop a deprenyl-derived enhancer substance that is free of the MAO-B inhibitory property (Knoll et al. 1992a). (–)-1-Phenyl-2-propylaminopentane [(–)-PPAP] has been chosen as our reference substance with this pharmacological profile.

Figure 3.6 shows the chemical structure and pharmacological spectrum of PEA and its four most representative synthetic derivatives.

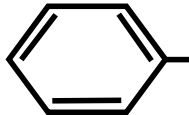
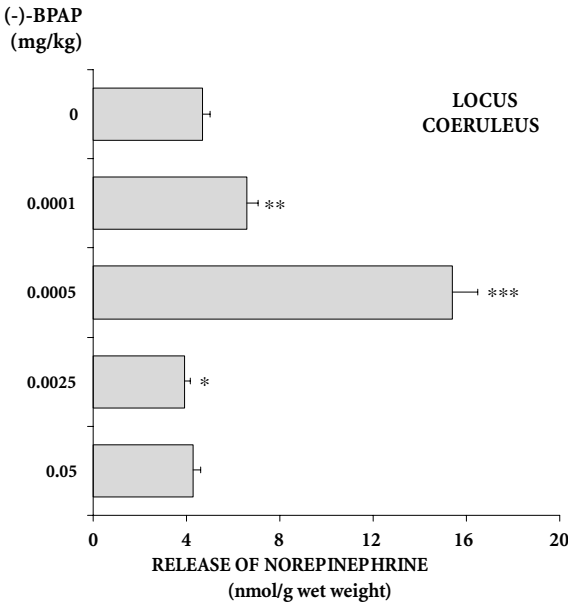
				ENHANCER EFFECT	RELEASING EFFECT	RELATION TO MAO
β -PHENYLETHYLAMINE (PEA)	CH ₂ - CH	- N		+	+	SUBSTRATE TO MAO-B
	H	H	H			
AMPHETAMINE	CH ₃	H	H	+	+	WEAK MAO INHIBITOR
METHAMPHETAMINE	CH ₃	CH ₃	H	+	+	WEAK MAO INHIBITOR
(-)-1-PHENYL-2-METHYL- N-METHYL-PROPARGYL- AMINE, (-)-DEPRENYL	CH ₃	CH ₃	C ₃ H ₃	+	0	POTENT MAO-B INHIBITOR
(-)-1-PHENYL-2-PROPYL- AMINOPENTANE, (-)-PPAP	C ₃ H ₇	H	C ₃ H ₇	+	0	0

Fig. 3.6. The chemical structure and pharmacological spectrum of PEA and its most representative synthetic derivatives. Taken from Knoll 2001

Fig.3.7. Significant enhancement of norepinephrine release from the locus coeruleus of rats isolated 30 min after the subcutaneous administration of a single dose of (-)-BPAP. The amount of norepinephrine released from the tissue within 20 min following the administration of different doses of (-)-BPAP was measured according to Knoll and Miklya (1995). Horizontal lines to the right of the graph bars show SEM. Paired Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001



3.1.3.2
(-)-BPAP, the Tryptamine-Derived Enhancer Substance

The discovery that tryptamine is also an endogenous enhancer substance (Knoll 1994) opened the way for a structure-activity relationship study aiming to synthesize a new family of enhancer compounds structurally unrelated to PEA and the amphetamines. *R*-(*-*)-1-(benzofuran-2-yl)-2-propylaminopentane [(*-*)-BPAP] was selected as a tryptamine-derived synthetic mesencephalic

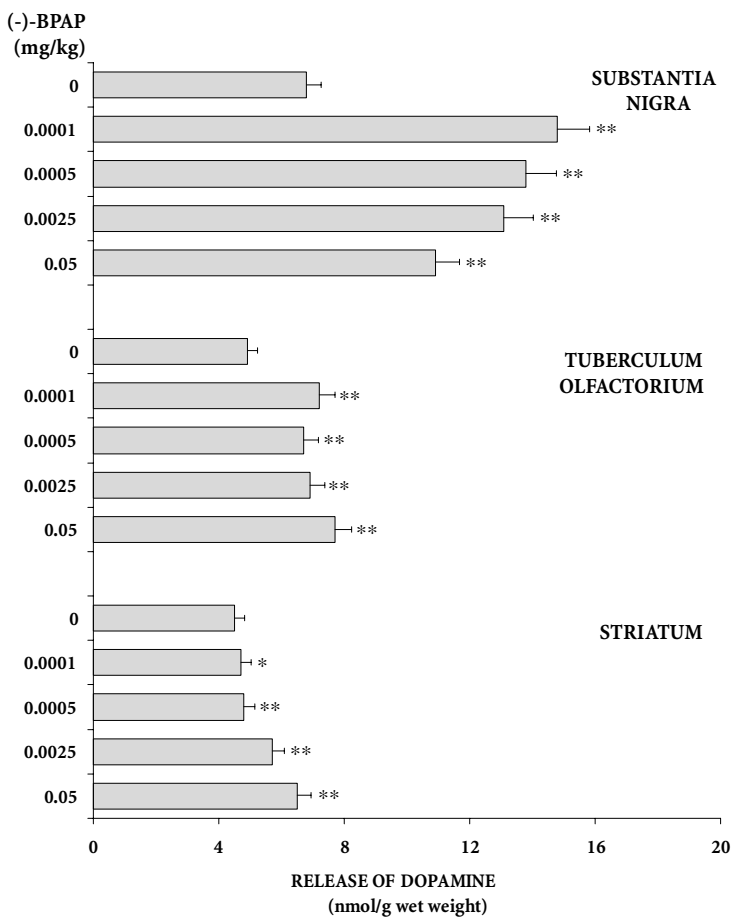


Fig.3.8. Significant enhancement of dopamine release from the substantia nigra, tuberculum olfactorium, and striatum of rats, respectively, isolated 30 min after the subcutaneous administration of a single dose of (-)-BPAP. The amount of dopamine released from the tissue within 20 min following the administration of different doses of (-)-BPAP was measured according to Knoll and Miklya (1995). Horizontal lines to the right of the graph bars show SEM. Paired Student's *t*-test. **P* < 0.05, ***P* < 0.01

enhancer compound for further studies (Knoll et al. 1999). For details of its chemistry see: Oka et al. (2001) and Yoneda et al. (2001).

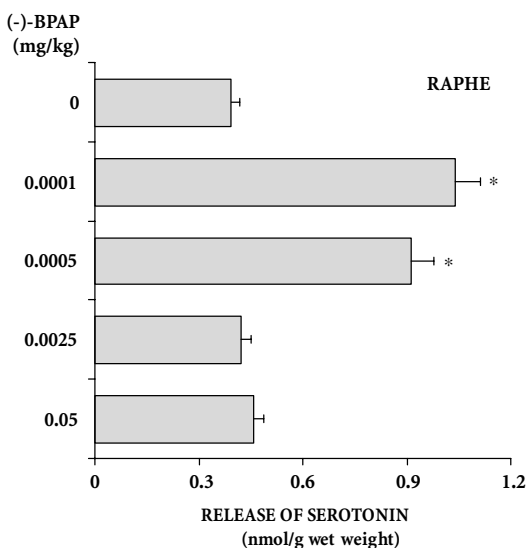
The *in vivo* dose-dependent enhancer effect of (–)-BPAP is illustrated on noradrenergic (Fig. 3.7), dopaminergic (Fig. 3.8), and serotonergic neurons (Fig. 3.9), respectively. A comparison of the enhancer effect of (–)-BPAP and (–)-deprenyl shows

1. The substantially higher potency of (–)-BPAP than (–)-deprenyl in enhancing the activity of catecholaminergic neurons
2. The characteristic dose-dependency of the enhancer effect of (–)-BPAP on noradrenergic (Fig. 3.7) and serotonergic neurons (Fig. 3.9), and
3. The highly potent *in vivo* enhancer effect of (–)-BPAP on serotonergic neurons (Fig. 3.9) and the lack of this effect on the part of (–)-deprenyl (Fig. 3.5)

In a study the effect of uptake inhibitors (desmethyylimipramine, fluoxetine), a selective MAO-A inhibitor (clorgyline), a selective MAO-B inhibitor (lazabemide), and dopamine receptor stimulants (pergolide, bromocriptine) – in comparison to the effect of (–)-BPAP – was measured on electrical-stimulation-induced release of labeled transmitters from the isolated brain stem of rats following labeling with [^3H]-norepinephrine or [^3H]-dopamine or [^3H]-serotonin by preincubation in transmitter stores. The study confirmed the selectivity of the enhancer effect of (–)-BPAP (Miklya and Knoll 2003).

Figure 3.10 shows the chemical structure and pharmacological spectrum of tryptamine and two of the tryptamine-derived synthetic mesencephalic enhancer substances.

Fig. 3.9. Significant enhancement of serotonin release from the raphe of rats isolated 30 min after the subcutaneous administration of a single dose of (–)-BPAP. The amount of serotonin released from the tissue within 20 min following the administration of different doses of (–)-BPAP was measured according to Knoll and Miklya (1995). *Horizontal lines to the right of the graph bars show SEM. Paired Student's t-test. *P < 0.01*



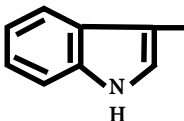
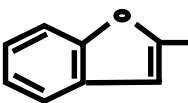
			ENHANCER EFFECT	RELEASING EFFECT	RELATION TO MAO
	CH ₂ - CH - NH				
TRYPTAMINE	H	H	+	0	SUBSTRATE TO MAO-A
(-)-1-(INDOL-3-yl)-2- PROPYLAMINO- PENTANE, (-)-IPAP	C ₃ H ₇	C ₃ H ₇	+	0	WEAK MAO-A INHIBITOR
	CH ₂ - CH - NH	C ₃ H ₇ C ₃ H ₇	+	0	WEAK MAO-A INHIBITOR
R-(-)-1-(BENZOFURAN-2-yl)-2-PROPYL- AMINOPENTANE, (-)-BPAP					

Fig.3.10. The chemical structure and pharmacological spectrum of tryptamine and two of its most representative synthetic derivatives. Taken from Knoll (2001)

3.2

Pharmacological Analysis of Mesencephalic Enhancer Regulation Using (-)-BPAP as a Specific Experimental Tool

3.2.1

Detection of a Specific and a Nonspecific Form of Enhancer Regulation in the Mesencephalic Neurons. Studies Using Isolated Discrete Rat Brain Regions

(-)-BPAP is at present the most selective and potent experimental tool to investigate enhancer regulation in the mesencephalon. The enhancer effect can be detected following the subcutaneous administration of low amounts of (-)-BPAP (see Table 2 in Knoll et al. 1999), as well as following the addition of the substance into the organ bath of freshly isolated discrete mesencephalic brain areas (see Table 3 in Knoll et al. 1999).

Enhancer substances stimulate the enhancer-sensitive neurons in the mesencephalon in a peculiar manner. Figure 3.11 shows the characteristics of the enhancer effect of (-)-BPAP added to isolated locus coerulei of rats. We see two bell-shaped concentration/effect curves. The one in the low nanomolar range, with a peak effect at 10^{-13} M concentration, clearly demonstrates the existence of a highly complex, specific form of enhancer regulation in noraadrenergic neurons. The second, with a peak effect at 10^{-6} M concentration, shows the operation of a ten million times less sensitive, obviously nonspecific form of the enhancer regulation in these neurons (see Knoll et al. 2002b, for details).

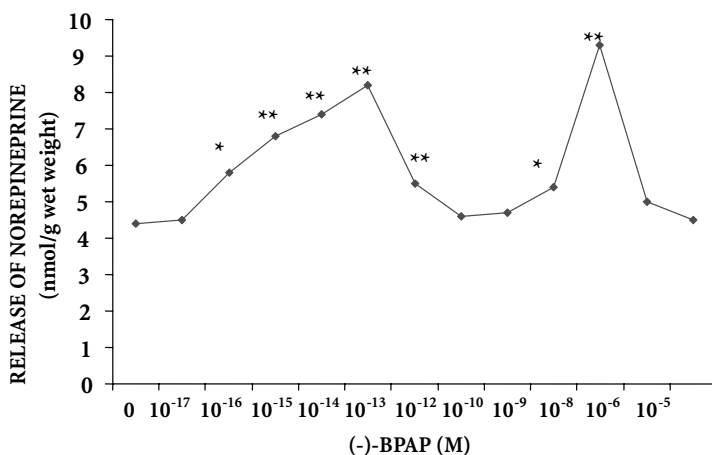


Fig. 3.11. The bi-modal, bell-shaped concentration effect curve characteristic to the enhancer effect of (-)-BPAP on isolated locus coerulei of rats. (-)-BPAP was given to the organ bath of the quickly removed locus coerulei. Eight organs were used for the analysis of each concentration. The amount of norepinephrine released within 20 min from the tissue in the presence of different concentrations of (-)-BPAP was measured according to Knoll and Miklya (1995). Paired Student's *t*-test. **P* < 0.01, ***P* < 0.001

We experienced, in a number of studies on rats (Knoll et al. 1955a,b,c, 1956, 1994; Knoll 1956, 1957, 1988) 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. As it will be further discussed later (see Sect. 4.1), the discovery of the bell-shaped concentration/effect curve of the enhancer substance in the low nanomolar concentration range offers the first reasonable explanation for the great individual variation in behavioral performances.

3.2.2

Analysis of the Two Forms of Enhancer Regulation on Isolated Brain Cells in Culture

(-)-BPAP also proved to be a proper experimental tool for detecting the presence and analyzing the nature of enhancer regulation on single brain cells in culture.

Considering the role of the mesencephalic neurons in goal-seeking behavior and collating this experience with the finding that the performance of the catecholaminergic and serotonergic neurons were significantly enhanced in rats *in vivo* with 0.0001 mg/kg (-)-BPAP (see Table 2 in Knoll et al. 1999) and *in vitro* at 10⁻¹³ M concentration (see Table 3 in Knoll et al. 1999), it

was reasonable to assume that this highly sophisticated form of enhancer regulation is the physiological mechanism in the mesencephalon responsible for a drive. We may also assume that from a physiological point of view the enhancement of nerve cell performance elicited by (–)-BPAP in the high concentration range is a non-specific effect, obviously unrelated to behavioral performances.

This view was substantiated by studies with (–)-BPAP on single brain cells in culture: (a) two studies on glial cells (Ohta et al. 2002; Shimazu et al. 2003), (b) a study on mesencephalic neurons (Knoll et al. 1999), and (c) two studies on cortical neurons (Hamabe et al. 2000, and see Figs. 3.12 and 3.13 in the present study).

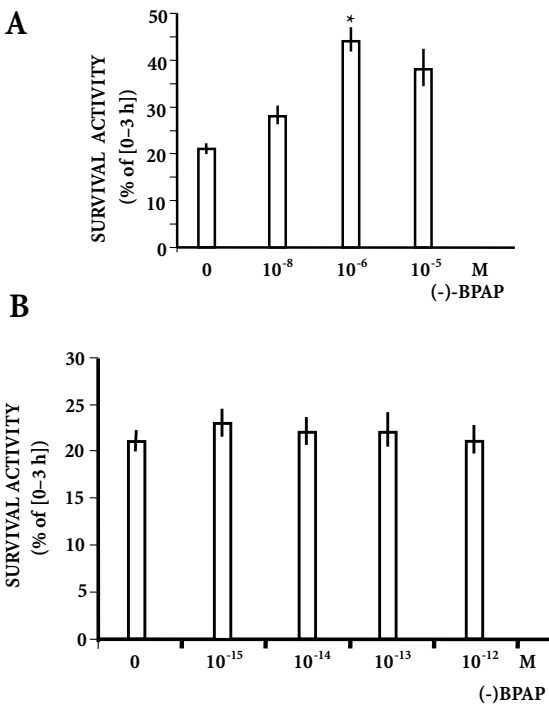


Fig.3.12. A Protective effect of (–)-BPAP in the high micromolar concentration range, with a peak effect at 10^{-6} M concentration, against serum-free condition induced cell death in low-cell-density culture of the cerebral cortex from E17 rats. B Lack of a protective effect of (–)-BPAP under the same conditions in the low nanomolar concentration range. Experiments were carried out in triplicate. Data are the mean \pm SEM from six independent experiments. The data were analyzed using Student's *t*-test after multiple comparisons of ANOVA. *P* value was < 0.05 compared with the results in the vehicle-treated culture. See Hamabe et al. (2000) for methodology

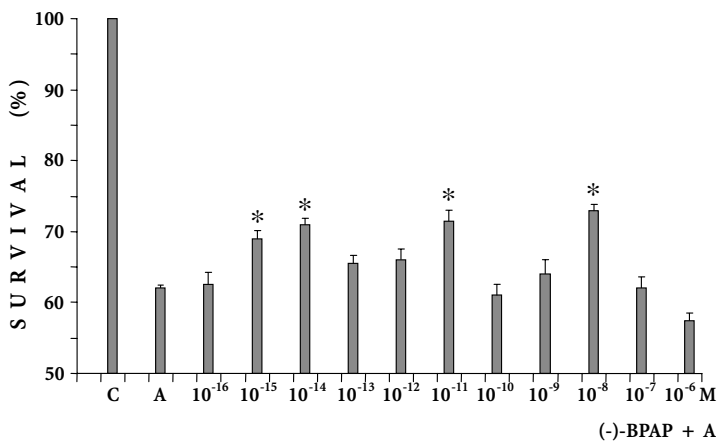


Fig.3.13. Protective effect of (–)-BPAP against β -amyloid_{25–35}-induced cell death on isolated cortical neurons from 8-day-old chicken embryos (Lohman brown hybrid). Duration of a single experiment: 10 days. (–)-BPAP has been added to the culture at the first day *in vitro*. Lesioning with β -amyloid_{25–35} pre-aggregated for at least 72 h. Concentration and stock solution 1 mM, lesioning with 10 μ l of the stock solution. C control, A β -amyloid_{25–35}. Graph bars given in percent of the unlesioned control (100%), represent the mean viability \pm SEM from two independent experiments performed on 2 days with two 96-well plates and six to eight identical wells/concentration and substance. Statistical analysis: two-tailed Student’s *t*-test for two means. **P* < 0.05

3.2.2.1
Studies on Cultured Neuroglial Cells

Neuroglia, the supporting structure of nervous tissue, consists of a fine web made up of modified ectodermic elements in which glial cells are enclosed. Neuroglial cells (astroglia, oligodendroglia, microglia) play an important physiological role in the brain and modulate the function of neurons in a complex manner. They do not participate, however, in the realization of drive-dependent goal-seeking behavior. Thus it was of crucial importance to test the effect of (–)-BPAP on the performance of glial cells. Two studies were performed with (–)-BPAP on cultured mouse astrocytes (Ohta et al. 2002; Shimazu et al. 2003).

As a quantitatively measurable specific function of glial cells, the rate of synthesis of three neurotrophic factors (nerve growth factor [NGF]; brain-derived neurotrophic factor [BDNF]; and glial cell line-derived neurotrophic factor [GDNF]) was measured. In the Ohta et al. (2002) study, the enhancer effect of (–)-BPAP was measured only in the high concentration range. The authors found the amount of NGF, BDNF, and GDNF secreted from astrocytes into the culture medium increased by up to 120, 2, and 7 times more, respectively, than those of the control treatment with 0.35 mM (–)-BPAP for 24 h. The (–)-BPAP-

induced increased production of NGF and GDNF was inhibited by concomitant administration of actinomycin D, given for transcription blockade. (–)-BPAP treatment increased the mRNA expression of NGF, BDNF, and GDNF. The results of this study proved that the nonspecific form of the enhancer regulation operates in glial cells.

In the second study the effect of (–)-BPAP was tested in a range of 10^{-15} to 5×10^{-4} M concentration. This study corroborated the finding of the first one. The synthesis of NGF was significantly enhanced in the high micromolar concentration range with a peak effect at 10^{-4} M concentration, whereas 5×10^{-4} M was ineffective. (–)-BPAP acted similarly on the synthesis of BDNF (with a peak effect of 10^{-4} M concentration) and on the synthesis of GDNF (with a peak effect of 10^{-4} M concentration) (Shimazu et al. 2003). But the crucially important step forward was the proof that, as expected, (–)-BPAP was ineffective in the low nanomolar concentration range. Thus the specific form of enhancer regulation was not detectable in glial cells.

This finding supports the view that the specific form of enhancer regulation stimulated by (–)-BPAP in the low nanomolar concentration range is the behaviorally important form, whereas the enhancer effect of (–)-BPAP in the micromolar concentration range is insignificant in behavioral terms. Nevertheless, the (–)-BPAP-induced enhancement of the synthesis of neurotrophic factors is a remarkable pharmacological effect whose therapeutic value deserves further analysis in the future.

3.2.2.2

Studies on Cultured Mesencephalic Neurons

The first analysis of the enhancer regulation on cultured neurons using racemic BPAP as a specific experimental tool was performed on rat hippocampal cells (Knoll et al. 1999).

To elicit cell death the cultured rat hippocampal neurons were treated with β -amyloid_{25–35}. BPAP (the racemic substance was used in this early study) exerted its enhancer effect in the characteristic bipolar manner, with bell-shaped concentration/effect curves. The peak effect was reached at 10^{-14} M concentration in the low nanomolar concentration range, and at 10^{-8} M concentration in the higher micromolar concentration range (see Fig. 5 in Knoll et al. 1999). Because of the neurotoxic effect of β -amyloid_{25–35}, no more than 20% of the cells, obviously the high performing cells, survived this attack. As the synthetic mesencephalic enhancer substance significantly enhanced the performance of the neurons in culture, in the presence of the optimum concentration (10^{-14} M) of BPAP about 70% of the cells survived.

(–)-BPAP enhanced the activity of the catecholaminergic and serotonergic neurons in isolated discrete mesencephalic regions in the exactly same bipolar manner and in the same concentration range (see Table 3 in Knoll et al. 1999).

The studies with (–)-BPAP performed on noradrenergic, dopaminergic, serotonergic and hippocampal neurons proved unequivocally the operation of a highly specific, complex form of enhancer regulation in the subcortical neurons. This is very much in keeping with the ascription of a commanding role to midbrain neurons in goal-seeking behavior.

3.2.2.3

Studies on Cultured Cortical Neurons

The first study of the enhancer effect on cultured cortical neurons was performed with (–)-BPAP on a primary culture of rat cerebral cortex. In this experiment the rapid cell death of the cortical neurons was measured in serum-free culture. It was shown that in a low-cell-density culture cortical neurons rapidly die. (–)-BPAP, as first shown by Hamabe et al., significantly protected the cortical neurons against serum-free-condition-induced cell death in the high concentration range (see Fig. 2 in Hamabe et al. 2000). The protective effect of (–)-BPAP, with a peak effect at 10^{-6} M concentration, is shown in Fig. 3.12A. However, in striking contrast to the finding on cultured rat hippocampal neurons (see Knoll et al. 1999, Fig. 5), (–)-BPAP did not exert an enhancer effect on the cultured rat cortical neurons in the nanomolar concentration range. This is shown in Fig. 3.12B.

To investigate the difference in sensitivity towards (–)-BPAP between the subcortical and cortical neurons of rats *in vivo*, we performed two series of experiments in the shuttle box.

Tetrabenazine treatment (1 mg/kg s.c.) depletes at least 90% of norepinephrine and dopamine from their stores in the nerve terminals of the catecholaminergic neurons and, 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 conditioned avoidance reflex (CAR). The learning deficit caused by tetrabenazine treatment can be antagonized by the administration of a synthetic mesencephalic enhancer substance.

In the shuttle box the acquisition of a two-way CAR was analyzed during 5 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 5 s, it was punished with an unconditioned stimulus (US), a footshock (1 mA). If the rat failed to respond within 5 s to the US, it was classified as an escape failure (EF). One trial consisted of a 15 s intertrial interval (IR), followed by 15 s CS. The last 5 s of CS overlapped the 5 s of US. At each learning session, the number of CARs, EFs and IRs were automatically counted and evaluated by multi-way ANOVA.

To test a compound's ability to enhance the acquisition of CARs in the shuttle box, it is necessary to select proper training conditions. In the case in which the rat was trained with 100 trials per day, the acquisition of CARs reached an 80% level and the EFs approached or reached the zero level. To demonstrate the highly significant enhancer effect of (–)-BPAP on the mesencephalic catecholaminergic neurons *in vivo*, we trained the rat with 100 trials per day, blocked the acquisition of CARs by pretreating the rats with tetrabenazine, and restored the learning ability with the simultaneous administration of (–)-BPAP. Table 3.1 shows that (–)-BPAP antagonized the effect of tetrabenazine in the rats.

Learning is a cortical function and in the series of experiments aiming to test the effect of (–)-BPAP on cortical neurons we trained the rats with 20 trials per day in order to have a chance to detect the drug-induced improvement in learning ability.

Table 3.1 shows that the percentage of CARs in rats trained with 100 trials per day was 77.13 ± 8.47 on the 5th day of the training (Series no. 1). In contrast, it was only 8.50 ± 2.47 in rats trained with 20 trials per day (Table 3.2, Series no. 1).

Table 3.1. Because of its enhancer effect on catecholaminergic neurons, (–)-BPAP antagonized tetrabenazine-induced learning deficit in rats trained in the shuttle box

Series no.	Compound (mg/kg)	Tetra-benazine (mg/kg)	Percentage of CARs	Percentage of EFs	Number of IRs
1	Saline (–)-BPAP	–	77.13 ± 8.47	6.00 ± 5.72	34.25 ± 11.21
2	–	1	5.00 ± 3.30	61.50 ± 13.80	5.83 ± 2.18
3	0.05	1	$46.88 \pm 14.15^*$	$17.88 \pm 9.30^{***}$	9.25 ± 2.81
4	0.10	1	$46.38 \pm 8.75^{***}$	$7.38 \pm 4.34^{****}$	6.75 ± 1.03
5	0.25	1	$59.00 \pm 12.62^{***}$	$5.25 \pm 2.13^{****}$	16.75 ± 5.74
6	0.50	1	$70.38 \pm 10.73^{****}$	$1.38 \pm 1.02^{****}$	8.50 ± 2.83
7	1.00	1	$87.75 \pm 1.95^{****}$	$0.13 \pm 0.13^{****}$	$27.38 \pm 4.49^{**}$
8	2.50	1	$79.75 \pm 7.03^{****}$	$1.38 \pm 1.12^{****}$	$24.50 \pm 9.19^*$
9	5.00	1	$92.00 \pm 2.47^{****}$	0.00^{****}	$57.88 \pm 19.37^*$
10	10.00	1	$92.00 \pm 2.46^{****}$	0.00^{****}	$68.33 \pm 26.46^*$

Tetrabenazine or the combination of tetrabenazine + (–)-BPAP was administered subcutaneously, 60 min before daily measurement.

Rats (in each group 4 males and 4 females) were trained at 100 trials daily for 5 days in the shuttle box. The performance on the fifth day of training is shown in the table.

CAR conditioned avoidance response; EF escape failure; IR intersignal reaction Significance of combination (tetrabenazine + (–)-BPAP) vs tetrabenazine (ANOVA):

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

Thus, in case (–)-BPAP had possessed a specific enhancer effect on cortical neurons, we could detect it easily in form of a significant, dose-dependent increase in the percentage of CARs and in the reduction of the percentage of EFs.

Because of the bell-shaped concentration effect curve characteristic to the enhancer effect of (–)-BPAP (see Fig. 3.11), we used 10 doses of the compound, ranging from 0.000001 to 10 mg/kg, to clarify the effect of (–)-BPAP on the cortical neurons. Table 3.2 demonstrates that none of the applied doses of (–)-BPAP was capable of changing the learning performance of rats in the shuttle box. In accord with the findings on cultured rat cortical neurons, the *in vivo* experiments confirmed that (–)-BPAP, the presently known most potent synthetic mesencephalic enhancer substance, is devoid of a specific enhancer effect on the cortical neurons.

The second study of the enhancer effect on cultured telencephalic neurons was performed on cortical cells from 8-day-old chicken embryos (Lohman brown hybrid). This is the only study to date on nonmammalian brain cells. (–)-BPAP detected the operation of both the specific and nonspecific form of enhancer regulation in the cortical neurons of this avian species. The performance of the cortical neurons was enhanced in the low nanomolar concentration range of (–)-BPAP, with a peak effect at 10^{–14} M concentration (Fig. 3.13).

Table 3.2. Because of its ineffectiveness on cortical neurons, (–)-BPAP did not enhance the learning performance of rats trained in the shuttle box

Series no.	(–)-BPAP (mg/kg)	Percentage of CARs	Percentage of EFs	Number of IRs
1	Saline	8.50 ± 2.47	0.75 ± 0.62	1.88 ± 1.01
2	0.000001	6.13 ± 1.99	1.25 ± 0.65	4.00 ± 2.65
3	0.00001	4.38 ± 2.08	3.25 ± 1.56	3.63 ± 1.46
4	0.00005	5.88 ± 2.44	2.38 ± 1.96	2.50 ± 1.02
5	0.0001	12.75 ± 2.18	0.63 ± 0.63	1.25 ± 1.68
6	0.0005	9.63 ± 2.07	0.63 ± 0.42	6.50 ± 3.08
7	0.025	8.50 ± 2.48	2.25 ± 1.16	2.88 ± 1.39
8	0.05	8.63 ± 2.13	0.00	4.38 ± 2.34
9	0.1	6.75 ± 2.96	2.13 ± 2.13	2.13 ± 0.61
10	1.0	8.50 ± 2.63	0.00	4.25 ± 1.82
11	10.0	0.63 ± 0.42	1.13 ± 0.74	2.88 ± 1.26

(–)-BPAP was administered subcutaneously, 60 min before daily measurement Rats (in each group 4 males and 4 females) were trained at 20 trials daily for 5 days in the shuttle box. The performance on the fifth day of training is shown in the table. CAR conditioned avoidance response; EF escape failure; IR intersignal reaction Significance of (–)-BPAP vs saline was calculated according to ANOVA; in all cases *P* > 0.05.

For a trial aiming to explain the striking sensitivity difference of the cultured cortical cells of rats and chickens towards (–)-BPAP see Sect. 3.4.1.

3.3

Considering Enhancer Receptors

According to our present knowledge substances that change the activity of a cell in very low concentrations exert their effect via a highly specific receptor. The finding that (–)-BPAP enhances the activity of the noradrenergic, dopaminergic, serotonergic and hippocampal neurons in the brain at 10^{-13} – 10^{-16} M concentration (Knoll et al. 1999) speaks in favor of the existence of highly specific enhancer receptors in these neurons.

In order to get direct evidence for (–)-BPAP-sensitive receptors, we performed experiments with [3 H]-(–)-BPAP. Unfortunately, we were unable to find unequivocal evidence for the predicted binding sites for [3 H]-(–)-BPAP. Furthermore, the explanation of the peculiar bell-shaped concentration/effect curve characteristic of the enhancer effect of (–)-BPAP (see Fig. 3.11) remains to be understood.

It is obvious that the enhancer effect of (–)-BPAP exerted, for example, on the isolated locus coeruleus of rats (see Fig. 3.11) with a peak at 10^{-13} M concentration is a highly specific effect from a physiological point of view, while the second peak at 10^{-6} M concentration represents a nonspecific effect of minor importance. The proposition that (–)-BPAP binds to a specific receptor in the technically unmeasurable concentration range and higher concentrations induce a conformational change which makes the binding of the ligand impossible would explain both the bell-shaped concentration/effect curve and the ineffectiveness of our trials to furnish unequivocal evidence for the binding of [3 H]-(–)-BPAP to its receptor. Nevertheless, the observed phenomena need clarification.

Because we were unable to find, using [3 H]-(–)-BPAP, direct evidence for specific enhancer receptors, we tried to approach the problem from another angle. In the rat brain, using a classic pharmacological method, we found convincing indirect evidence for (–)-BPAP-sensitive enhancer receptors in the mesencephalon (Knoll et al. 2002a).

1-(2-Benzofuryl)-2-(3,3,3-trifluoropropyl)-aminopentane HCl (3-F-BPAP), a close structural analogue of BPAP with weak enhancer activity, was synthesized with the expectation that the simultaneous administration of this analogue with (–)-BPAP would significantly antagonize the enhancer effect of the latter, proving that they act on the same receptor. The low specific activity of 3-F-BPAP was demonstrated in the rat in the shuttle box.

The subcutaneous administration of 1 mg/kg tetrabenazine depletes the catecholamine stores in the brain within 1 h. As a consequence of this change, tetrabenazine treatment inhibits the acquisition of a two-way avoidance reflex

in the shuttle box. This effect can be significantly antagonized by enhancer substances. The effect of (–)-BPAP was measured in eight different doses from 0.05 to 10 mg/kg. Even the lowest dose significantly antagonized tetrabenazine-induced inhibition of learning (see Table 3.1). In contrast, 3-F-BPAP was ineffective in five different doses, ranging from 0.25 to 5.0 mg/kg (Table 3 in Knoll et al. 2002a).

The concurrent administration of 1 mg/kg 3-F-BPAP with 0.1 mg/kg (–)-BPAP significantly inhibited the enhancer effect of (–)-BPAP, but 1 mg/kg 3-F-BPAP did not influence the enhancer effect of 1 mg/kg (–)-BPAP (Fig. 2 in Knoll et al. 2002a). This is a clear indication that the compounds bind to the same receptor, to which (–)-BPAP has a much higher affinity than 3-F-BPAP.

(–)-Deprenyl, at present the only enhancer drug in general use, though being substantially less potent in the shuttle box than (–)-BPAP, significantly antagonized the learning deficit caused by tetrabenazine. We studied the effect of 1 and 5 mg/kg (–)-deprenyl in different combinations with 1 and 5 mg/kg 3-F-BPAP and found that 3-F-BPAP left the enhancer effect of (–)-deprenyl unchanged (Fig. 3 in Knoll 2002a). Furthermore, 3-F-BPAP did not influence the enhancer effect of (–)-PPAP, the (–)-deprenyl analogue free of MAO-B inhibitory potency (Fig. 4 in Knoll 2002a).

The data prove that the molecular mechanism through which the PEA-derived substances, (–)-deprenyl and (–)-PPAP, exert their enhancer effect *in vivo* is not identical with the mechanism through the stimulation of which the tryptamine-derived substance, (–)-BPAP, acts. This is in accord with the finding that, in contrast to (–)-BPAP, (–)-deprenyl did not exert an enhancer effect on the serotonergic neurons (see Fig. 3.5, and for more details Knoll et al. 1999). That (–)-BPAP enhances the activity of the catecholaminergic and serotonergic neurons in the rat brain via the stimulation of a highly specific enhancer receptor is strongly supported by the finding that the compound did not show a significant binding capacity to any of the receptors known to play a role in the function of the catecholaminergic and serotonergic neurons (see Table 3 in Knoll et al. 1999).

The characteristic enhancer effect of (–)-BPAP, as shown for example in Fig. 3.11, in the low nanomolar range and at a higher micromolar range (Knoll et al. 1999, Yoneda et al. 2001) indicate the existence of two types of (–)-BPAP-sensitive enhancer receptors in the brain stem neurons represented by high- and low-affinity binding sites. The recent identification of a family of G-protein-coupled trace-amine receptors in the mammalian brain specifically stimulated by the endogenous enhancer substances, PEA and tryptamine (Borowsky et al. 2001), strongly suggests that the authors located a family of enhancer receptors. This assumption is suggested by the finding that amphetamine and metabolites of the catecholamines neurotransmitters were also found to be antagonists of a rat trace-amine receptor (Bunzow et al. 2001).

The obvious difference already established between the binding of (–)-deprenyl and (–)-BPAP (Knoll et al. 1999) argues for the existence of various types of enhancer receptors. Remarkably, Borowsky et al. (2001) found that more than one member of the newly identified family of mammalian G-protein-coupled receptors was activated by PEA and tryptamine.

Studies with (–)-BPAP, the most potent and selective synthetic mesencephalic enhancer substance, which is presently also the best experimental tool for the analysis of the binding of enhancer substances to receptors, are at the very beginning. Nevertheless, two studies have already been published showing that (–)-BPAP has remarkable binding capacity to some receptors. Hamabe et al. (2000) demonstrated that high concentrations of (–)-BPAP displaced the binding of [³H]-(+)-pentazocine to sigma receptors in the synaptic membranes from rat cerebral cortex. Thereafter, Rashid et al. (2001) has found that (–)-BPAP binds to metabotropic sigma receptors in peripheral nociceptor endings. The sigma agonist-induced nociception was found to be due to the release of substance P from nociceptor endings through activation of Gα_{i1} and phospholipase C (Ueda et al. 2000). A number of studies indicated that sigma agonists stimulate heterometric G-proteins (Connick et al. 1992; Tokuyama et al. 1999; Maruo et al. 2000). The nociceptive flexor responses in mice induced by both (+)-pentazocine and (–)-BPAP were blocked by sigma receptor antagonist BD 1063. In radio-ligand binding assay, [³H]-(+)-pentazocine showed a saturable specific binding in membrane preparation from mouse liver, and this specific [³H]-(+)-pentazocine binding was inhibited by (–)-BPAP as well as by (+)-pentazocine and BD 1063 (Rashid et al. 2001). According to our present knowledge it is hard to find any reasonable relation between the binding of (–)-BPAP to the sigma receptors and its enhancer effect.

Not only the real nature of the specific mesencephalic enhancer receptors but also the endogenous ligands to these receptors remain unresolved. The high potency of (–)-BPAP in comparison to the already identified natural enhancer substances, PEA and tryptamine, is remarkable. This difference gives justification for the search for much more potent natural enhancer substances than PEA and tryptamine.

3.4

Cortical Enhancer Regulation: Assumptions About Its Physiological Significance

3.4.1

Essential Forms of the Modification of Behavior Through Exercise, Training, or Practice

The remarkable difference in sensitivity for (–)-BPAP between the isolated cortical cells of rat and chicken (see Sect. 3.2.2.3) is worthy of particular

attention. (–)-BPAP exerted its enhancer effect with a peak at 10^{-14} M concentration on isolated cortical neurons from 8-day-old chicken embryos, but was ineffective in the nanomolar concentration range on a primary culture of rat cerebral cortex. Although the experimental tool used for eliciting cell-death was β -amyloid_{25–35} in the chicken experiment (Fig. 3.13) and serum-free condition in the rat study (Fig. 3.12), it seems unreasonable to make the applied methods responsible for the observed difference. It seems much more plausible that we are dealing with a basic functional difference between the cortical neurons of the two species: *rats possess the ability to acquire drives, chickens are devoid of it.*

The faculty for acquiring a drive is uncommon in the animal kingdom. It was shown by Berta Knoll in the late 1950s that the mouse, a rodent closely related to the rat, was unable to acquire the glass-cylinder-seeking drive (B. Knoll, Thesis, 1968). She has found that, in striking contrast to the rat, the mouse was even unable to fix the inextinguishable form of the CAR, the functional stage that preceded the acquisition of the glass-cylinder-seeking drive in the rat (B. Knoll 1961).

In the initial training phase leading to the manifestation of the glass-cylinder-seeking drive, the rat was forced – for a couple of weeks, three times daily, on 10–50 occasions – to jump, when pushed through the side opening of a glass cylinder standing on a metal plate heated to 60 °C, on to the upper rim of the glass cylinder. The rat's behavior was modified after a short training period. The animal soon escaped from the unheated plate within 10 s, even 100 times in succession. The acquired inextinguishable CAR remained stable without reinforcement.

Trained under the same experimental conditions, the mouse seemed to behave similarly to the rat. To the end of the daily experiment the mouse escaped from the unheated plate within 10 s, even 100 times in succession. However, in striking contrast to the rat, the mouse was unable to fix the acutely detectable modification of behavior in the cortex. The next day there was no sign of the acquisition of a CAR. Even weeks of daily training did not modify this behavior of the mice. Thus, the experiments furnished unequivocal evidence for a qualitative difference in the natural endowments of the cortical neurons of mice and rats. The rat brain possesses the ability to fix chains of ICRs, while the mouse brain did not reach this stage of development.

From a physiological point of view the brains of members of the same strain are undeniably equal in their natural endowments. The device is the same. In the human, however, the extreme differences in life conditions that primarily determine the realm of the acquired drives, combined with substantial individual differences in learning ability, make unpredictable as to which trifling proportion of the immense inborn functional pool will be utilized. An individual necessarily strives to build those forms of acquired drives that demand the shortest training time with the lowest investment of energy. It is the plastic

description of this phenomenon that if their life conditions allow it, individuals aspire to select their activities according to their abilities.

To translate this description into the language of neurochemistry, we may say that it is the natural endowment of individuals to give preference to activities according to the efficiency of the enhancer regulation in the population of cortical neurons responsible for the selected performance. The best performing, the *talented* individuals will be the ones who mobilize when needed the specific enhancer substance at the optimum concentration (see Sect. 3.2.1).

We compared, in our longitudinal studies on rats, innate (hunger and sexual) drives with an acquired (glass-cylinder-seeking) drive. We arrived at the conclusion that the modification of behavior through practice, training, or experience means that groups of neurons acquire the ability to change the functional state of other groups which thereafter enables their cooperation. The sequence of learning-induced, exactly measurable modifications of behavior in the rat allows us to define four functionally different states of cortical neurons, the chemistry of which remains to be clarified. Accordingly we distinguish four functionally different groups of cortical neurons:

Group 1. Cortical neurons in their inborn naive state. Neurons are born to perceive special senses (light, color, sound, smell, taste, pain, touch) and when stimulated, an evoked potential is detectable in the especially sensitive group of cortical neurons within 0.015 s. The mammalian brain is supplied with such a high number of cortical neurons that, considering the short lifetime of the organism, a high percentage of the cortical neurons probably preserve their inborn functional state.

Group 2. Cortical neurons whose functional state has been modified through learning to serve an ECR. Pavlov's discovery that the main condition for the acquisition of an ECR is the stimulation of a special group of cortical neurons with their specific stimulus *simultaneously* with the precipitation of an unconditioned reflex can still hardly be overestimated. This type of behavioral modification has certainly been the most thoroughly studied phenomenon in the history of brain research. In our experiments the physiological significance of this functional state was illustrated by the quick adaptation of the glass-cylinder-seeking rats, which generate and extinguish long chains of ECRs (tool reflexes) according to need, enabling them to easily reach the goal in spite of unforeseeable changes in the environment (Knoll 1969). Mainly *followers of Pavlov* tried for a while to maintain the false doctrine that the totality of higher nervous activity can be explained through this mechanism.

Group 3. Cortical neurons whose functional state was modified through learning to serve an ICR. We experienced the fixation of chains of ICRs in the course of the training procedure aiming to develop the glass-cylinder-seeking drive in rats. It was shown in Daniel Bovet's laboratory by Kelemen et al. (1961) that an ICR can sharply be differentiated by EEG from an ECR. It seems obvious that in every moment of life the already firmly fixed, readily ecphorizable stock of

chains of ICRs represents the consciously perceivable, stable knowledge of the individual.

Group 4. Cortical neurons whose functional state was modified through learning to serve an acquired drive. A population of cortical neurons (the cortical representation of the drive) acquires, through proper training, the ability to produce, when needed, the specific enhancer substance in an optimum concentration, reach the highest possible level of excitability and stay in this state continuously ('active focus') until the goal is reached. Drives determine the life of the mammalian organisms as their operation is the condition *sine qua non* for the building, fixing, and ecphorizing chains of ECRs and ICRs whenever required.

For a domesticable mammal ready to acquire drives, life means the practice-, training-, or experience-induced continuous transition of the functional state of cortical neurons in the above-cited sequence from Group 1 to Group 4. As a consequence of these changes, we observe the proper modification of behavior. The human brain is, of course, the best model for studying this chain of events.

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A healthy human brain possesses, practically speaking, an immense capacity to fix chains of ICRs and acquired drives. This is obvious when we call to mind, as an example, the fact that the human brain is born with billions of neurons belonging to Group 1 that just serve the sense of hearing. If we follow this train of thought and restrict ourselves only to one aspect of this basic brain function, the ability to fix chains of ICRs belonging to the world of music, it is easy to realize that this primarily hearing-dependent capacity of the human brain is by itself inexhaustible. And this is all the more so, since the chains of ICRs can also be fixed by reading the proper notes.

It depends of course on living conditions which small proportion of disposable neurons will definitely ascend in the hierarchy and thus assume the role of Group 3 neurons during the lifetime of the individual. We know that whatever human activity is measured we observe extreme individual differences in performance due to substantial individual differences in the cortical enhancer regulation (see Sect. 4.1). But, in the course of the short human lifetime, even a talent on the order of magnitude of a Johann Sebastian Bach can utilize only a humble proportion of the neurons available for fixing chains of ICRs in the field of music. This is true of all kinds of human activities, since the human brain possesses a network of over 100 billion interrelated nerve cells and a 10^{10} bit capacity.

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A human cortex is, at birth, comparable with a book consisting of billions of empty pages. Life is obviously too short to scribble over this entire book. Every mammalian organism can be defined at any given moment by the number of

neurons that have already changed their functional state by this date. In the case of human beings the self is determined by the already fixed chains of ICRs and acquired drives, as their operation, in contrast to the function of chains of ECRs, is inseparable from conscious perception.

Domestication of animals proves that even in ancient times humans had recognized the ability of some animal species to acquire drives for unnatural goals and made a good use of it. It is reasonable to assume that the essential cortical mechanism responsible for the transition of a naive neuron in a sequence of events until the acquired drive is fixed is the same when a rat acquires the glass-cylinder-seeking drive or a human fixes any form of an acquired drive. Nevertheless, there can be no doubt that human performance is qualitatively different from the performance of a domesticable animal. We may interpret this difference as a typical example of the transition of quantity into quality. There is an enormous quantitative difference between the most clever animals and humans in the ability of their naive cortical neurons to change their functional state and ascend in the hierarchy until they ultimately become part of Group 3 or 4. In striking contrast to the cortex of the domesticable animals, the human cortex is capable of making this alteration with ease and high speed. Compared to humans, the ability of the brain to fix chains of ICRs and to acquire a new drive is a rudimentary function even in the anthropoid apes.

In our studies aiming to build the glass-cylinder-seeking drive into the brain of rats, we trained hundreds of animals and followed their performance until they died (Knoll et al. 1955a,b,c, 1956; Knoll 1956, 1957). In one series of experiments on a random group of 100 two-month-old rats (50 males, 50 females) we developed – within a 3-week training period in each animal – the inextinguishable conditioned jumping reflex. Yet out of the hundred rats, only 20% of the population (11 females, 9 males) showed clear-cut signs of a tendency to acquire the glass-cylinder-seeking drive and only two of them (one male and one female) ultimately maintained this drive through their entire life. If we compare the restricted ability of rats to build acquired drives to the almost unlimited ability of the human brain to acquire new drives, the qualitative difference in performance is understandable without any need to deny that the mechanism of the cortical enhancer regulation is essentially the same in the two species. This is simply a new example of the general rule in nature that an immense variety of colorful phenomena, in this case the fantastic variation in the outward form of behavioral performances, rests on the operation of a common simple mechanism. Somewhat like “Gravitation keeps the whole universe going.”

Although any form of an acquired drive is rooted in one of the innate drives, as soon as the new drive develops and operates in an inextinguishable manner, the roots become unrecognizable. Watching a glass-cylinder-seeking rat in operation, one cannot recognize that escape from a hot plate was the foundation of this acquired drive.

It seems reasonable to assume that, from a functional point of view, the appearance of species with the ability to acquire drives for unnatural goals was the last radical turning-point in the development of brain organization. In the animal kingdom the new mechanism reached its functionally most sophisticated level in the group of anthropoid apes, but it reached perfection in *Homo sapiens* only. The new mechanism culminated in the development of speech – the classic, human-specific instrument that made interpersonal communication possible by capturing reality in the form of symbols – and thus opened the way for the operation of an unrestricted variety of acquired drives.

The learning of each letter is in itself the fixation of a chain of ICRs. The learning of each word represents the fixation of a much more complicated chain of ICRs in which the sequence of letters is determinant. The words will then be used as tools to build sentences. A sentence is part of an acquired drive induced goal-oriented behavior. The words are used as rapidly changeable tools for reaching a practically infinite number of goals. Speech, in conjunction with all other forms of language-based interpersonal contacts, brought forth the most sophisticated technique in service of goal-oriented behavior and produced the highest level of human achievements, science and art. This method allowed, with greater or lesser efficiency, the preservation of the achievements of ancestors, leading to the uniqueness of human society. In this society each generation stands on the shoulders of past generations, evaluating history and envisaging the future.

*

To understand the past and envisage the future of the human society we should never forget that the last step forward in the development of life on earth, the evolution of brains with the ability to acquire drives for goals unnecessary for the survival of the individual or the species, is based on a cortical mechanism that humans share with a couple of animal species. An “active focus” is created in the brain through learning: a population of neurons ascend into Group 4.

The operation of the acquired-drive-directed behavior means, objectively, the proper chemical changes in the cortical representation of the drive, subjectively, the imagination (mental representation) of the goal to be reached. As a matter of fact, the operation of those neurons in the brain that have learned to cooperate with each other and represent an integral whole also represents the proper cognitive/volitional and affective consciousness which we simply describe as the imagination of the goal to be reached. In practice, when the specific enhancer substance is produced in its optimal concentration and keeps the neurons belonging to the “active focus” at their highest level of excitability, the urge starts operating and the individual is ready to conquer any obstacle to reach the subjectively imagined goal.

From the point of view of the basic physiological mechanism of acquired-drive-directed behaviors there is no difference between a glass-cylinder-

seeking rat and a scientist or artist who seeks to reach a special, highly sophisticated goal. *Only the goals to be reached are qualitatively different.* Using the same mechanisms, the rat is looking for a glass cylinder, the creative scientist for something previously unknown, and the creative artist for something previously nonexistent.

The creative human mind is the best example for understanding the essential characteristics of acquired-drive-directed behavior. For sake of illustration we may take as an example an immortal achievement of the human brain, the unusually well-documented birth of *Composition VII*, the most monumental oil painting by Kandinsky, a master for whom the creation of a work of art was the creation of a world.

Kandinsky executed the painting between November 25 and 28, 1913. His pupil and life-partner, Gabriele Münter, took four photographs in the span of 3 1/2 days, documenting the progress of the work. On the other hand, 33 works (drawings, watercolors, oil paintings) related to this composition are known. Kandinsky, who wished to demonstrate that color is as expressive and powerful as sound in making art without narrating anything realistic, irrefutably proved his thesis with *Composition VII* (first exhibited in 1914 in Cologne). This is truly a breathtaking symphonic construction in painting.

Collating the analysis of the 33 related works (Dabrowsky 1995) with Gabriele Münter's photos showing the progress of the work until the oil painting was finished, we see, in a highly sophisticated form, the trial and error mechanism that always operates until a goal is reached. The acquired drive, the "active focus", subjectively the imagination of the glass cylinder, drove our rats until the goal was reached; and the acquired drive, the "active focus", subjectively the imagination of *Composition VII*, drove Kandinsky until his goal (now permanently exhibited in the Tretyakov Gallery in Moscow) was reached.

Basic laws are simple, gray. The phenomena brought into existence by them are, however, immensely complex and colorful. With the billions of functional units in the brain capable of cooperating with each other, a simple mechanism, cortical enhancer regulation, brings into existence an immense variety of colorful acquired-drive-directed behaviors. Glass-cylinder seeking is an example of the simplest forms of such behavior, while production of *Composition VII* represents one of the most sophisticated, breathtakingly complex forms of acquired-drive-produced behaviors.

3.4.2

The Concept that Learning Is a Cortical Enhancer Regulation Dependent Function

In vertebrates, learning – the modification of behavior through practice, training, or experience, one of the essential necessities of life – is the main physiological function of the cortex. Modification of behavior rests upon the inborn ability of cortical neurons to get acquainted with each other through training,

learn to influence each other's function, and cooperate thereafter according to need. The mechanism of this important process is, however, still unknown. The discovery of enhancer regulation offers the following interpretation of learning.

Each member of a population of naive cortical neurons (Group 1) born to perceive a specific quality of stimuli, originating from outside or inside the body, synthesizes the same enhancer substance. It is also supplied with enhancer receptors for which this enhancer substance is the highly specific ligand. The stimulation of the neurons with their enhancer substance leads to enhanced excitability. On the other hand, each cortical neuron is able to activate under proper conditions (training) an enhancer receptor to any of the existing cortical enhancer substances (learning). Thus, *neuron A* is born with its specific enhancer receptor (ER_A) and with the ability to synthesize its own enhancer substance (ES_A). *Neuron B* is born with ER_B and synthesizes ES_B , and so on. *Whenever a cortical neuron gets excited, its specific enhancer substance is synthesized in an increased amount, and its sensitivity toward other enhancer substances is significantly increased.*

When neurons A and B are simultaneously stimulated, both are continuously bombarded with a higher amount of the enhancer substance of the other neuron and at the same time also sensitized to activate a receptor to the alien enhancer substance. As a consequence, the concurrent stimulation of neurons A and B time after time (training) ultimately leads to the fixation of a new functional constellation. Neuron A acquires sensitivity toward ES_B , and neuron B acquires sensitivity toward ES_A . Thus, learning means that a neuron acquires the ability to respond to originally alien stimuli. As a consequence of this change we experience the training induced modification of behavior.

Using the shuttle box technique, there is a reasonable possibility of testing the validity of this concept on rats. The shuttle box is a simple and useful setup for following the development of a two-way conditioned avoidance reflex (CAR). The box is divided inside into two parts by a barrier with a small gate. The rat is trained to cross the barrier under the influence of flash light (conditioned stimulus, CS). If the rat fails to do so, the animal is punished with an electric footshock (unconditioned stimulus, US). The rat is trained with 100 trials/day. One trial consists of a 15 s intertrial interval, followed by 15 s flash light that overlaps with a footshock for 5 s. If the rat does not cross the barrier to footshock within 5 s, this is noted as an escape failure (EF). The rat learns to avoid punishment and escapes in response to flash light within 10 s (CAR). The percentage of CARs and EFs as well as crossings during the 15 s intertrial interval (intertrial response, IR) is automatically registered.

According to present views, the rat, driven by fear, tries to prevent punishment and learns by trial and error to escape in due time. The efficiency of learning is thought to be proportional to the number of the successful crossings in response to flash light within 10 s. According to our new concept the

efficiency of learning depends on the repeated simultaneous operation of functionally different populations of cortical neurons. In the light of this approach we need to weigh carefully the series of events in the cortex during the training procedure.

The concept predicts that the development of a stable CAR in the shuttle box signifies the acquisition of a special cooperation between the groups of cortical neurons born to perceive the footshock (US) and the flash light (CS), respectively. Nevertheless, other groups of cortical neurons (stimulated, e.g., by the setup as a whole) are also involved in the special modification of the rat's behavior. In the course of training numerous groups of cortical neurons, A, B, C ... n, born to perceive special information only, are synchronously active and influence each other. Furthermore, each group of neurons has the chance to develop sensitivity toward each of the enhancer substances belonging to the simultaneously activated groups of neurons. Thus, during the training procedure a network of cooperating groups of cortical neurons develops, which operates thereafter as an entity. The training-induced cooperation between the groups of neurons can be 1. transient in nature (chain of ECRs), 2. irreversibly fixed (chain of ICRs), or 3. may lead to the development of the most sophisticated form of excitatory state in a group of cortical neurons ("active focus") that will operate thereafter as an acquired drive. However complicated the cooperation developed between different group of neurons during training may be, it is their common feature that they work thereafter as an integral whole, and this entity can be activated via a few decisive groups of neurons.

Humans, capable of communicating via symbols, can easily experience the operation of this mechanism. Each letter, a basic symbol of communication, is by itself a chain of ICRs fixed forever in the brain when reading/writing was learned. Each word is a much more complicated chain of ICRs based on the special sequence of letters. A sentence is an acquired-drive-induced goal oriented function that uses words as tools.

A word consisting of, let's say, eight letters is a chain of eight chains of ICRs that are fixed in a special sequence, and the totality of this complicated system is perceived as a whole. Whenever the word is ecphorized as a whole, this means the explosion-like activation of the groups of cortical neurons in the sequence as they were fixed when we learned the word.

As it will be discussed in detail later (see Sect. 4.2), it is enough to see the first, last and one or two intermediate letters to activate the whole chain of the irreversibly fixed eight chains of ICRs in their special sequence and perceive the word as an entity *with the same speed* as the correctly written one. If these letters are left untouched, all the words of a long sentence can be similarly misspelled without interfering with comprehension of meaning. In case of a higher degree of confusion of the letters, a longer time is needed until the word as an integral whole can be consciously ecphorized. Based on

the experience of this well-known ability of the human cortex, it has become a popular TV quiz all over the world to present the completely jumbled letters of a longer word or a short sentence, with the prize going to the contestant who most quickly recognizes the word or phrase.

According to our approach learning means the establishment of a cooperation between functionally different groups of cortical neurons (Group 1), born to perceive only one special type of stimulation. In response to proper training, however, they also learn to respond to an alien stimulus. Thus whenever we ecphorize the acquired engram, we simultaneously activate functionally heterogeneous groups of cortical neurons. It depends on the quality of training whether an untrained cortical neuron (Group 1) ascending in the hierarchy assumes the role of those of Group 2 (ECR) Group 3 (ICR), or Group 4 ("active focus"), but only the proper activation of Group 3 and 4 neurons is inseparable from conscious perception.

It is obvious that much more time is needed for the activation of functionally heterogeneous groups of cooperating neurons than for the activation of a functionally homogeneous group of neurons. This has already been unequivocally proven in humans (Libet 1973), although the reason for the observed phenomenon remained unexplained until now.

Libet performed a series of experiments on fully conscious patients during the exposure of a cerebral hemisphere for some neurosurgical procedure. The postcentral gyrus was electrically stimulated with extreme care in order to establish the conscious perception of this stimulation.

By definition, only the proper stimulation of neurons belonging to Group 3 or 4 is inseparable from conscious perception. Here we have to remember even an excellently operating glass-cylinder-seeking rat that was brought to the laboratory every day lingered for a longer period of time before it started to work. We described this phenomenon as "warming up" (see Knoll 1969, for review). It seems reasonable to assume that a longer time is needed until the enhancer regulation in the cortical neurons belonging to Group 4, that operate as the "active focus," "the cortical representation of the drive," is transformed to the state at which production of the enhancer substance reaches the critical concentration and the neurons arrive at the level of excitability, subjectively to the "imagination of the glass cylinder," which is the precondition for the readiness to go through fire and water to reach the goal.

Protocols 1, 2 and 3 (Sect. 4.2) provide examples of the "warming-up" phenomenon. Protocol 1 shows, for example, that on March 4 the rat reached the goal in 12'01" at the first trial, in 4'29" at the 2nd trial, and this time varied between 57" and 2'35" in the following eight trials. The rats whose performance is registered in Protocols 2 and 3 behaved similarly.

Libet found that a single stimulus was ineffective. For the conscious perception of cortical stimulation he needed to apply trains of 0.5-ms pulses at liminal intensity for as long as 0.5-s duration.

Thus Libet has detected experimentally in humans the phenomenon we observed in rats and described as “warming up.” He just did not know what happened. He obviously activated, with the aid of electrical stimulation, a chain of ICRs and ecphorized the engram as an integral whole. He needed 0.5 s until the cooperating neurons were brought to the state of excitability inseparable from conscious perception.

Libet found that the same long period was required for the sensory perception of a cutaneous stimulation. He stimulated the skin of the hand with a brief electrical pulse. Though 0.015 s is enough to elicit an evoked potential in the somesthetic cortical area in response to stimulation, *once again 0.5 s elapsed before the skin stimulation was consciously perceived.*

This means that in the Libet experiment a period of time 33 times longer (0.5 s) was needed for the activation of the functionally heterogeneous groups of cooperating cortical neurons that learned to work together in the past, ascended in the hierarchy and assumed the role of Group 3, than for the activation of a functionally homogeneous group of naive cortical neurons (0.015 s).

With all this in mind, our approach was that the modification of behavior of the rats trained in the shuttle box depends on the synchronous activation of different groups of cortical neurons in the brain for a proper period of time. The following method is suitable to test the validity of this approach.

Treatment of rats with 1 mg/kg tetrabenazine, which blocks selectively and reversibly the reuptake of the catecholaminergic transmitters into their intraneuronal stores, depletes norepinephrine and dopamine from the end organs of the catecholaminergic neurons in the brain stem. Since the operation of the catecholaminergic brain engine is the condition *sine qua non* for the trial-and-error mechanism and thus for the success of reaching a goal, the acquisition of a CAR in the shuttle box cannot be detected in tetrabenazine-treated rats because of the blockade of the animal's ability to cross the barrier.

Nevertheless, the activation of the cortical neurons via the US and CS remains unchanged in tetrabenazine-treated rats, as can be shown by measuring the evoked potentials following stimulation in the proper cortical area. Thus, according to our concept, the condition in the shuttle box for learning must be unchanged in tetrabenazine-treated rats. To be able to detect whether rats learn when pretreated with tetrabenazine, we performed the experiments on Charles River Wistar Wistar rats which, according to our earlier experiments, proved to be a strain with exceptionally low learning capacity in the shuttle box (Knoll et al. 1996c). We used females as they are even worse performers than males.

In the first part of the experiment (Series A), groups of female rats ($n = 8$) were trained in the shuttle box from Monday until Friday with 100 trials/day for 5 consecutive weeks. The animals were treated subcutaneously, 1 h prior to measurement, either with 1 ml/kg saline (Group 1) or with 1 mg/kg tetrabenazine (Group 2). Following a 5-week training period (Series A), the animals were rested for 3 weeks and then trained again for 3 consecutive weeks (Se-

ries B). The rats in Group 1 were injected daily with saline. The animals in Group 2 were treated daily with 1.5 mg/kg tetrabenazine during the 1st and 2nd week and with saline during the 3rd week.

To illustrate the changes in behavior during the first training period, Table 3.3 shows the performance of the rats on the 1st and 5th day of the 1st and 5th week (Series A). The saline-treated rats (Group 1) developed a stable CAR by the end of the training period. Flash light, the CS, was – with an average of $78.88 \pm 11.92\%$ – effective in eliciting the escape of the rats to the other part of the compartment within 10 s. The efficiency of footshock practically reached its maximum. The EFs dropped to an average of $1.90 \pm 1.63\%$. *Treatment with 1 mg/kg tetrabenazine (Group 2) inhibited the performance of the rats significantly.*

As was discussed above, to have a better chance to see whether rats are capable of learning when the catecholaminergic system in the mesencephalon is blocked by tetrabenazine treatment, we were compelled to work in this series of experiment with a strain of low performing (“dull”) rats. Especially the females of this strain of rats need a few weeks to fix a stable CAR. Table 3.3 shows that on the 1st day of training only $10.50 \pm 4.69\%$ of the conditioned stimulations were effective and by the end of the 1st week only an average of $48.88 \pm 9.30\%$ was reached. Under the same training conditions, the rats of a high performing (“clever”) strain are capable of escaping in response to flash light on the 1st day of training in more than 80% of the trials (as an example see Knoll et al. 1996b). Table 3.3 also shows that females in this “dull” strain of rats needed a 5-week training period to reach the level of performance that “clever” rats already produce on the 1st day of training.

The saline-treated rats (Group 1) fixed a stable CAR during the 1st training period. In the 2nd training period (Series B), that started after 3 weeks of rest, the CS elicited the escape of rats on the 1st day of training at an average of $82.85 \pm 1.86\%$, and the EFs in response to electric shock reached the zero level ($0.95 \pm 0.38\%$).

In Group 2 we raised the dose of tetrabenazine to 1.5 mg/kg after the 3-week resting period, and we treated rats for 2 weeks with this high dose. This treatment blocked almost completely the rats’ ability to respond to outside stimuli. On the 5th (last) day of the 2nd week of treatment, there was no sign of conditioned avoidance (average of CARs: $3.50 \pm 2.28\%$) and the average percentage of EFs mounted to 63.13 ± 14.31 . Before starting the 3rd week of training on Monday, the rats had their usual rest for two days (Saturday/Sunday). This was sufficient time for the elimination of tetrabenazine and the refilling of the catecholamine stores in the mesencephalic neurons.

During the 3rd week of training the rats of Group 2 were treated with saline. The performance of the rats on the 1st and 5th days are shown in Table 3.3. *The rats treated with tetrabenazine for 7 weeks and trained, produced the same average of CARs ($80.75 \pm 10.29\%$) as their saline-treated peers ($80.79 \pm 2.05\%$).*

Table 3.3. The modification of behavior of tetrabenazine-treated rats through training in the shuttle box

Series A	Week of training	Performance on the	Group 1. (Control) saline treatment for 5 consecutive weeks		Group 2. Tetrabenazine treatment (1 mg/kg) for 5 consecutive weeks	
			CAR (%)	EF (%)	CAR (%)	EF (%)
3-week stop in training	1 st	1 st day	10.50 ± 4.69	22.50 ± 7.55	5.00 ± 2.76	65.00 ± 12.29*
		5 th day	48.88 ± 9.30	3.00 ± 2.37	27.63 ± 12.12	49.00 ± 14.92**
	5 th	1 st day	63.79 ± 13.80	1.55 ± 1.33	20.00 ± 9.59*	56.38 ± 15.56**
		5 th day	78.88 ± 11.92	1.90 ± 1.63	29.00 ± 11.61*	46.38±16.92**
Tetrabenazine treatment (1.5 mg/kg) for 2 consecutive weeks						
Series B	1 st	1 st day	82.85 ± 1.86	0.95 ± 0.38	8.25 ± 5.76***	56.50 ± 12.46**
		5 th day	83.40 ± 1.68	0.98 ± 0.42	1.88 ± 0.99***	66.00 ± 13.04**
	2 nd	1 st day	83.00 ± 1.52	1.00 ± 0.58	1.50 ± 0.85***	65.88 ± 12.89**
		5 th day	82.05 ± 1.82	0.95 ± 0.60	3.50 ± 2.28***	63.13 ± 14.31**
Saline treatment						
3 rd	1 st day	1 st day	80.79 ± 2.05	1.20 ± 0.63	80.75 ± 10.29	10.00 ± 9.29
		5 th day	84.50 ± 1.75	1.15 ± 0.53	86.25 ± 1.73	0.75 ± 0.41

Two groups of female rats ($n = 8$) stemming from a strain with very low innate learning capacity were treated in the shuttle box at 100 trials daily for 5 days weekly. The rats were treated subcutaneously 60 min before training with saline and tetrabenazine, respectively. Detailed explanation in the text. *CAR* conditioned avoidance response; *EF* escape failure. Significance in the performance between the saline-, and tetrabenazine-treated rats was evaluated by multi-factor analysis of variance (ANOVA): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

On the 5th day of the 3rd week of training in Series B, there was no difference even in the EFs between the two groups. It is obvious that rats treated with tetrabenazine learned similarly to the saline-treated rats, we were just unable to detect this modification of behavior because mesencephalic enhancer regulation was blocked in Group 2 by tetrabenazine treatment, and the rats were unable to operate in the shuttle box.

The results are in accord with the concept that learning needs only the concurrent operation of functionally different groups of cortical neurons under proper conditions. As the circumstances for the cortical neurons were the same from the beginning until the end in the saline-, and tetrabenazine-treated groups of rats, the modification of behavior was also the same.

The data are in harmony with the working hypothesis that each functionally homogeneous group of cortical neurons is continuously producing its own highly specific enhancer substance, the amount of which is significantly increased when the neuron is activated via its specific stimulus. Considering that the most potent synthetic mesencephalic enhancer substance, (–)-BPAP, exerts its enhancer effect in a range of 10^{-16} – 10^{-14} M concentration (Knoll et al. 1999), it is reasonable to assume that the cortical enhancer substances also work in a very low concentration. This may throw unusual technical difficulties in the way of detecting and identifying the key actors in cortical enhancer regulation.

3.5

Therapeutic Aspects of Synthetic Mesencephalic Enhancer Substances

3.5.1

The Physiological Mechanisms that Give Reason for the Prophylactic Administration of a Synthetic Mesencephalic Enhancer Substance to Slow Brain Aging

An almost sateless agility, playfulness, and exuberant high spirits after weaning that is extinguished when sexual maturity is reached and a slowly developing decline of behavioral performances during the downhill period of life characterize the brain work of mammalian species capable of acquiring drives.

Our studies on rats suggest that age-related changes in mesencephalic enhancer regulation are primarily responsible

1. For the youthful power of the mammals from weaning until sexual maturity
2. For the transition from the uphill period of life into postdevelopmental longevity
3. For the progressive decay of behavioral performances during the downhill period of life, and
4. For the transition from life to death

Hundreds of millions of people now die at ages over 80, primarily due to the twentieth century progress in hygiene, chemotherapy, and immunology. With a longer average life span the need to improve quality of life during the latter decades of life is more compelling.

It is not to be questioned that brain aging, the unavoidable age-related decay of brain performances, gives a lot of trouble to the aged, causes hardly tolerable inconveniences, and too often makes the latter decades of life a burden. It is therefore hard to overestimate the significance of a safe and efficient prophylactic treatment that slows the aging of the brain.

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The belief that a steadily acting toxic agent, oxygen, is to blame for age-related deterioration of systems with aerobic metabolism is as simple as it is attractive. Oxygen is a Janus-faced substance: on the one hand, essential for living; on the other, toxic in nature. The cells which use oxygen to maintain their organization and viability use their scavenger systems to fight incessantly against toxic free radicals generated from oxygen. The idea that the life of living beings with aerobic metabolism is ultimately terminated by chronic oxygen toxicity seems to be, at first sight, self-evident and is substantially supported by the shorter life span of species with a higher metabolic rate.

It is common knowledge, however, that cells of vital organs, including the brain, maintain vigorous activity at natural death. The unavoidable chronic oxygen toxicity helps us to understand the progressive, age-related decay of organ function, but cannot explain why natural death sets in exactly at the time it does. *The riddle to be solved is: why does the organism as a whole die, when the aged organs remain fit for life at natural death, even though the passage of time causes deterioration of parts of the system?*

As the brain alone ensures that the mammalian organism works as a purposeful, motivated, goal-directed entity, without denying the significance of the adverse consequences of natural aging in different organs, we may assume that none of them can compete in importance with age-related changes in the CNS.

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 within broad limits according to need. Such regulation is the condition *sine qua non* for the integrative work of the CNS. The operation of the catecholaminergic system is comparable to an engine which is ignited once for an entire lifetime, as signaled by the appearance of an EEG, in an early phase of development.

Due to aging, the maximum level of activation of the CNS, via the catecholaminergic system, decreases progressively with the passing of time. The blackout ("natural death") of the integrative work of the CNS, signaled by the disappearance of an EEG, occurs when the catecholaminergic system's abil-

ity to activate the higher brain centers sinks below a critical threshold and an emergency incident transpires, where 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 challenge easily surmountable given catecholaminergic machinery working at full capacity may cause death in old age.

The essence of this hypothesis is depicted in Fig. 3.14. According to this scheme, the life of a mammalian organism can be divided, from a functional point of view, into six stages, each beginning with a qualitative change of crucial importance. The first stage starts with the fertilization of the ovum and lasts until the catecholaminergic system properly activates the higher levels of the brain, which then take the lead and integrate the different parts of the organism into a highly sophisticated entity. We may deem the first stage of development of the mammalian organism as completed when the catecholaminergic engine of the brain 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 to 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.

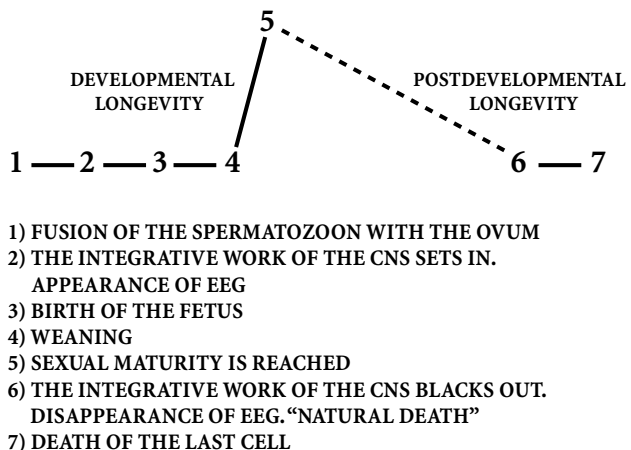


Fig. 3.14. Conception of essential changes during the lifetime of mammals

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 fulfils 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, postdevelopmental stage of life (aging) begins.

The essence of the fifth stage is progressive decay of the efficiency of the catecholaminergic system during the postdevelopmental life span until 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 an EEG signal, sets in.

As the parts of the organism remain alive, the sixth and last stage of life is the successive dying off of the different groups of cells.

The hypothesis outlined suggests that the quality and duration of life rests upon the inborn efficiency of the catecholaminergic brain machinery, i.e., a high-performing longer-living individual has a more active, more slowly deteriorating catecholaminergic system than its low-performing, shorter-living peer. To simplify the concept, we may say that a better brain engine allows better performance and a longer life span. *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 into a better-performing, longer-living one. It therefore follows that a shift of the duration of life beyond the technical life span (TLS), with a yet unpredictable upper limit, must be possible in all mammals, including the human species.*

Various species live together on earth in a harmonious proportion. This is obviously carefully regulated. One of the seemingly principal regulatory mechanisms that produces equilibrium among living organisms is brain aging. It ultimately leads to the elimination of those individuals who have already fulfilled their duty of nurturing the new generation.

Now we have to realize that the uphill period of life is epitomized by the operation of the enhancer regulation that maintains the basic activity of the brain on a significantly higher level (Knoll and Miklya 1995). The period of enhanced activity lasts until sex hormones appear, dampen enhancer regulation, and lower the basic brain activity to its preweaning level (Knoll et al. 2000). Thus, sex hormones provide for the transition from the developmental phase of life to postdevelopmental longevity, the period of the slow age-related decay of brain performance and terminated by natural death.

Although the slow and continuous age-related decline of enhancer regulation (the *vis vitalis*) that is characteristic of the downhill, postdevelopmental phase of life starts with the full-scale development of sexual-hormone regulation, it does not mean that the sexually mature individual is immediately converted to a significantly lower performer in its fight for existence. As it was shown earlier in detail, conditioning (learning) makes the performance of the experienced organism highly economic and efficient, even at a lower level of specific activation of the brain (Knoll 1969). Nevertheless, the irresistible, progressive age-related decay of enhancer regulation gradually weakens the compensatory role of experience, and even the most experienced aged organism becomes more and more vulnerable with the passing of time.

Considering the key role of the mesencephalic catecholaminergic neurons in the age-related deterioration of behavioral performances, it stands to reason that to fight against this unavoidable physiological process, there is a need to start a specific prophylactic therapy against brain aging as soon as sexual maturity has been reached. It seems logical that the best chance to realize this aim is the daily small dose administration of a synthetic mesencephalic enhancer substance during the downhill period of life. To justify this conclusion the brain mechanisms that constitute the basis of a prophylactic antiaging therapy need to be surveyed in more detail.

3.5.1.1

Developmental Longevity and Its Termination by Sex Hormones

How can youth be defined; how long does it last; how is it terminated? Or, using a more scientific sounding terminology: *what is the essential difference between developmental and postdevelopmental longevity; what is the cause of the transition from one phase to the other?*

To answer these questions we need to consider a phenomenon of which we first took notice in the course of our behavioral studies on rats performed in the 1950s. We observed that hunger drive induced orienting-searching reflex activity was significantly more pronounced in young rats than in their elder peers (Knoll 1957). We repeatedly corroborated this observation later and described it for the last time in 1995 (Knoll and Miklya 1995.)

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

Figure 3.15 shows the striking difference in the intensity of orienting-searching reflex activity of hungry rats in surroundings quite new to them as a function of time elapsed from last feed. Rats in their uphill period of life

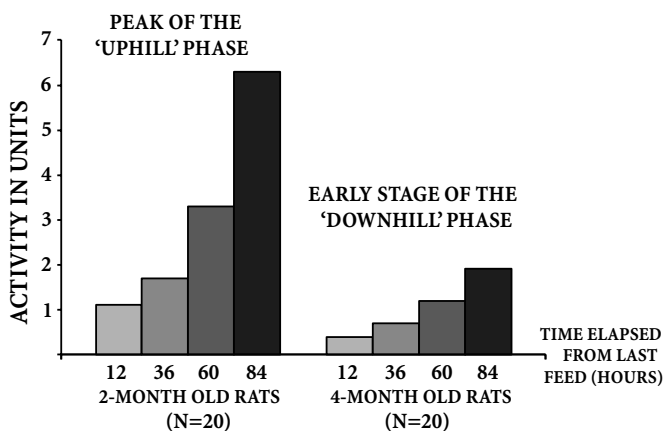


Fig.3.15. Intensity of orienting-searching reflex activity of hungry rats in surroundings quite new to them as a function of time elapsed from last feed. Activity was measured and expressed in units from 0 to 10. See Knoll and Miklya (1995) for methodology and other details

were much more active than their peers in their early postdevelopmental phase of life.

We have also followed the awakening of sexual drive, maturation of spermatozoa and development of the penis in male CFY rats. In the strain we used in this experiment, it was exceptional to find copulatory drive manifesting in males younger than six weeks. Although the appearance of copulatory patterns usually precedes maturation of spermatozoa and full development of the penis, the overwhelming majority of the males reached full-scale sexual activity by the completion of their 2nd month of life.

3.5.1.1.1

Enhanced Mesencephalic Enhancer Regulation from Weaning Until Sexual Maturity: The Mechanism Responsible for the Exuberant Physical Strength and Mental Vigor in the Uphill Period of Life

In the rat, the interval from weaning (3rd week of life) until the end of the 2nd month of age is decisive for the development of the individual. During this period the animal acquires abilities crucial for survival and maintenance of the species. Based on the observation that 2-month old hungry rats are significantly more active than their 4-month old peers, we checked 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 postdevelopmental 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

release of dopamine from the striatum, substantia nigra and tuberculum olfactorium, of norepinephrine from the locus coeruleus, and of serotonin from the raphe, in male and female rats (Knoll and Miklya 1995).

We found that from weaning until the 2nd month of life the striatal dopaminergic system of the rats was significantly more active than either before or after that period. This explains why, as demonstrated in Fig. 3.15, hungry rats in their developmental phase of life were significantly more mobile in an open field than their peers in their postdevelopmental phase of life. Similar age-related changes were detected in the mesolimbic dopaminergic, noradrenergic, and serotonergic systems, too. As an example, Fig. 3.16 illustrates, in male rats, the significantly enhanced release of catecholamines from the mesencephalic catecholaminergic neurons after weaning. Fig. 3.17 shows, in male rats, the dampening of the enhanced mesencephalic enhancer regulation after sexual maturity was reached.

The amount of serotonin released in 4-week-old rats was 7-fold higher in males and 6-fold higher in females than in 2-week-old animals. Thereafter, the amount of serotonin released from the raphe decreased with time, but remained significantly higher than in 2-week-old rats. In contrast, the amount of dopamine released from the striatum or tuberculum olfactorium of 16- to 32-week-old rats did not differ from that measured in 2-week-old animals. The amount of norepinephrine released from the locus coeruleus of 16- to 32-week-old rats was significantly lower than that in 2-week-old animals.

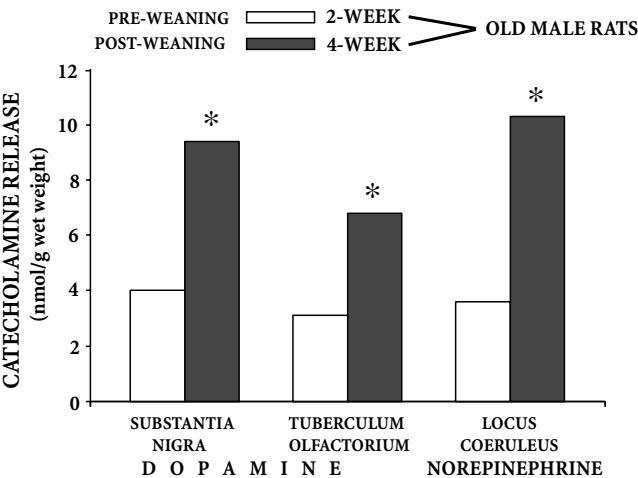


Fig.3.16. Proof of enhanced catecholaminergic activity in the brain stem of sexually immature rats after weaning. * $P < 0.001$. See Knoll and Miklya (1995) for methodological details

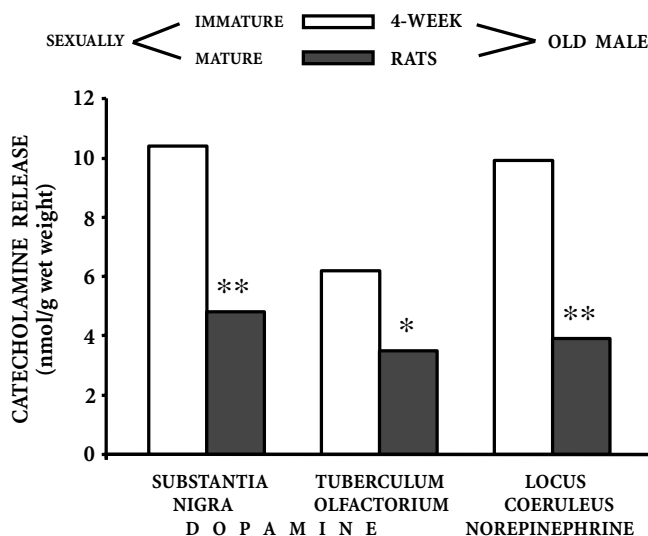


Fig.3.17. Proof of dampened catecholaminergic activity in the brain stem of rats following sexual maturity. * $P < 0.01$; ** $P < 0.001$. See Knoll and Miklya (1995) for methodological details

All in all, we found that 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 postdevelopmental (aging) phase of life, and we may also conclude that enhanced 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.

3.5.1.1.2

Sex Hormones Bring Back the Enhanced Mesencephalic Enhancer Regulation to the Prewaning Level: The Mechanism that Terminates Developmental Longevity and Constitutes the Foundation of the Transition From Adolescence to Adulthood

As it was shown earlier (Knoll and Miklya 1995), 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 sex hormones (end of the 2nd month of life).

Examples of these characteristic changes are shown in Figs. 3.16 and 3.17. Sexual hormonal regulation starts working in the rat with full capacity only

at the end of the 2nd month of age. The rapid decrease of the release of norepinephrine, dopamine and serotonin from selected discrete brain regions appeared synchronously with the completion of sexual maturity. Thus it was reasonable to assume that sex hormones dampen the enhancer regulation in the brain stem, and this is the mechanism which terminates developmental longevity.

We castrated three-week-old male and female rats and measured the release of catecholamines and serotonin from selected discrete brain regions at the end of the third month of their life (Knoll et al. 2000). Figure 3.18 shows, for example, that in male rats the amount of catecholamines and serotonin 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.

To further analyze this effect of sex hormones, we treated male and female rats s.c. 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 enhancer regulation. Twenty-four hours after a single injection with the hormones, the release of norepinephrine, dopamine and serotonin was significantly inhibited in the testosterone-, or estrone-treated rats, but remained unchanged after progesterone treatment. 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

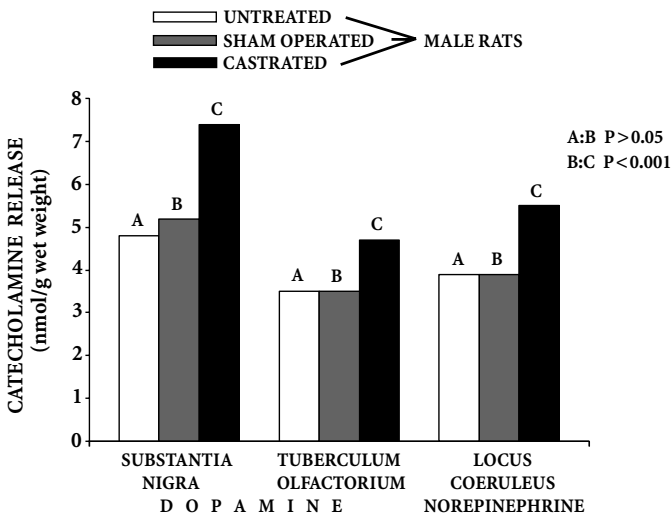


Fig. 3.18. Proof of enhanced catecholaminergic activity in the brain stem of 3-month-old rats castrated at the age of 3 weeks. See Knoll et al. (2000) for methodological details

rats treated with daily hormone injections for 7 or 14 days. After two-week treatment with the hormones, estrone was in the male and testosterone in the female the significantly more potent inhibitor of the enhancer regulation. Figure 3.19 shows, as an example, the dampening of enhancer regulation in male rats treated for 2 weeks with sex hormones.

The data prove that sex hormones terminate the hyperactive phase of life by dampening enhancer regulation in the catecholaminergic and serotonergic neurons. They bring about the transition from the developmental phase of life to postdevelopmental longevity, from adolescence to adulthood. This change is in the meantime also the beginning of the slow, continuous decay of mesencephalic enhancer regulation. As a consequence of it, the fixation of ICRs and the acquisition of drives are subject to an irresistible, slowly progressing, age-related decline until death.

Although individual variation in the age-related decline of behavioral performances is substantial, the process hits every brain. Both the decay in brain performances as well as the potential for the manifestation of age-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 prophylactic pharmacological intervention, starting immediately after the completion of sexual maturity, can significantly slow brain aging.

To find, sometime in the future, efficient means to prolong human life beyond the TLS would be a new example of man's endeavor to outwit Nature by understanding the laws of its operation.

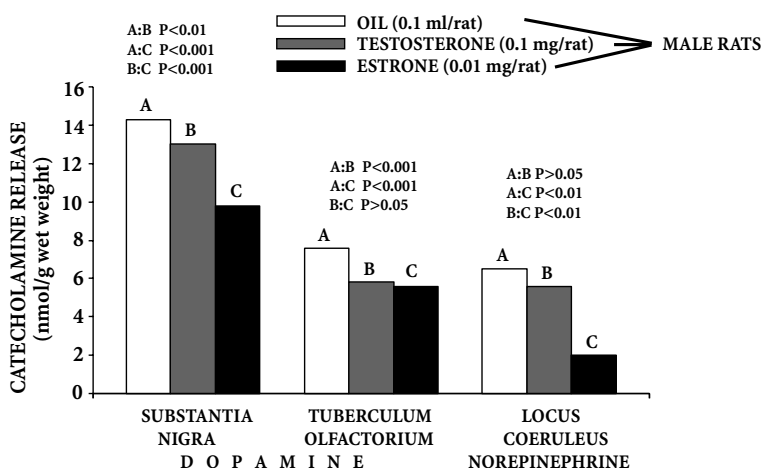


Fig.3.19. Proof of dampened catecholaminergic activity in the brain stem of 5-week-old rats following a 2-week treatment with sex hormones. See Knoll et al. (2000) for methodological details

3.5.1.2

Postdevelopmental Longevity and its Termination by Natural Death

Aging, the unfortunate common fate of all mature adults, is a physiological phenomenon. It essentially means the decadence of the quality of life with the passage of time. The easily recognizable, external appearance of aging (graying hair, wrinkling skin, use of reading glasses, etc.) gives some information about the chronological age of the person, but these signs are not necessarily in complete harmony with the physiological age of the organ systems, with the measurable decrements of integrated functions (maximum O₂ capacity, maximum breathing capacity, maximum work rate, etc.) or with the almost unmeasurable mental deterioration.

The exact measurement of the age-related changes in man remains difficult because the most reliable technique for following the changes in a given individual over his or her entire life span is practically unfeasible. The available information about the age-related changes in human population stems from cross-sectional studies, from the comparison of differences in performances between different age groups.

The main problem is, however, that the scatter within a particular age group for any measurable parameter is extreme. The reason for this extreme variation is the lack of a general factor of physiological age. In cross-sectional studies no single age emerges as the point of sharp decline in function. Any individual may show different levels of performance and the careful observer finds many dissociations between “chronological” and “physiological” age. A 70-year-old man, for example, may retain sexual performance equal to that of 40-year-olds, but display a cardiac output equivalent to his peers and a visual memory equivalent to average values for 80-year-old subjects. An additional factor which explains the extreme difficulties in measuring age-related changes with statistical methods lies in the nonlinear nature of the regression of a number of functions. Despite all these weaknesses, the average life span in the most developed countries has already exceeded the 80-year level. This change has come about due to the prevention of premature deaths owing to the development of hygiene, immunology and chemotherapy. The technical life span (TLS) of the human race, close to 120 years, has remained, however, unchanged.

*

It was discussed above (Sect. 3.5.1) that

1. Since the brain alone ensures that the mammalian organism works as a purposeful, motivated, goal-directed entity, the age-related changes in the CNS are of particular importance.
2. Since the enhancer-sensitive neurons in the brain stem work as the engine of the brain, the slow, continuous, postdevelopmental functional decline of

mesencephalic enhancer regulation is of primary importance in the maintenance of the well-balanced equilibrium among living organisms, because it helps to eliminate the individuals who already fulfilled their duty in nurturing the new generation.

For the time being the prestigious task – the maintenance of mesencephalic enhancer regulation during the postdevelopmental phase of life on the enhanced level characteristic of developmental longevity – cannot be fully accomplished. However, it is already feasible to modestly slow the age-related decay of the catecholaminergic and trace-aminergic tone in the brain via the prophylactic administration of 1 mg (–)-deprenyl daily.

The development of (–)-BPAP, an at least hundred times more potent synthetic mesencephalic enhancer substance than (–)-deprenyl, is by itself a hint that our present knowledge about mesencephalic enhancer regulation is in a very early stage. The high potency of (–)-BPAP indicates already that much more potent natural enhancer substances than PEA and tryptamine might exist. Better understanding of mesencephalic enhancer regulation promises to develop more efficient techniques in the future to slow brain aging and prolong human life beyond the TLS.

3.5.1.2.1

The Slow Decline of Mesencephalic Enhancer Regulation from Sexual Maturity Until Death: The Mechanism of Brain Aging Primarily Responsible for the Downhill Period of Life

To get an overall picture of the slow, continuous functional decline of the mesencephalic enhancer regulation during the postdevelopmental phase of life we need to follow the age-related decay in the state of supply of the mammalian brain with important endogenous substances related to this regulation. Let us set out on the track of PEA and dopamine.

To date PEA is the most carefully investigated natural enhancer substance (see Sect. 3.1.2). The first note in the literature furnishing indirect evidence that PEA may be an endogenous CNS stimulant in humans was the finding of Fischer et al. (1968). They found that the urinary excretion of free PEA was reduced in depressed patients and suggested that a PEA deficit may be one of the biochemical lesions leading to depression. Experimental evidence was soon presented that PEA is an endogenous constituent of the mammalian brain (Fischer et al. 1972; Saavedra 1974; Wilner et al. 1974). Sabelli and Mosnaim (1974) expounded the hypothesis that PEA might play a physiological role in affective behavior.

Papers discussing the possible role of PEA as a physiological mood elevator (Greenshow 1989; Davis and Boulton 1994; Sabelli and Javard 1995; Sabelli et al. 1986, 1996; Premont et al. 2001) as well as papers proposing a role of trace amines in a series of illnesses, such as schizophrenia, depression,

attention deficit/hyperactive disorder, Parkinson's disease, Rett's syndrome, migraine, phenylketonuria, hepatic encephalopathy, and hypertension (Udin and Sandler 1976; Boulton et al. 1988; Saavedra 1989; Walker et al. 1996; Janssen et al. 1999; Sato et al. 2000; Premont et al. 2001) were continually published.

An important step forward in the history of trace amines was the discovery of the presence of high-affinity binding sites for tyramine, tryptamine, and PEA (Hauger et al. 1982; Nguyen and Juorio 1989; Nguyen et al. 1989). As already previously mentioned, the binding sites have been identified as G-protein-coupled trace-amine receptors (see Sect. 3.3); the expression of trace-amine receptor mRNA in human amygdala further suggests a role of trace amines in depression and anxiety disorders (Borowsky et al. 2001); and methamphetamine, the PEA-derivative with a long-lasting effect, is also a trace-amine receptor agonist (Bunzow et al. 2001).

The discovery that PEA is a natural enhancer substance (see Chap. 3) clarified the mechanism of the stimulatory effect of PEA and finally assigned this trace amine its physiological role in the regulation of behavioral performances (see Shimazu and Miklya 2004, for review). Long before the discovery of the enhancer effect of PEA (Knoll et al. 1996c), we had already established that during postdevelopmental longevity there is a continuously increasing PEA deficit in the mammalian brain (Knoll 1982). This paper put forth the thesis that the progressive decrease in brain catecholamines and trace amines is an unavoidable biochemical lesion of aging. This concept was based, on the one hand, on the enhanced MAO-B activity in the aging brain, and on the other hand, on the antiaging effect of (–)-deprenyl, the first highly potent and selective inhibitor of MAO-B described.

As a rule, enzyme functions decrease in the brain with the passing of time. B-type of MAO is an exception. Robinson et al. (1971, 1972) published the first papers demonstrating that MAO activity progressively increases in the aging brain. This finding was corroborated within a couple of years by different groups (Nies et al. 1973; Mantle et al. 1976; Shih et al. 1979; Carlsson 1979; Eckert et al. 1980; Fowler et al. 1980a,b; Strolin Benedetti and Keane 1980). It soon became clear, however, that both in the brain of humans (Fowler et al. 1980b) and rats (Mantle et al. 1976; Strolin Benedetti and Keane 1980) only the activity of the B-type of MAO is increased in the aged. It was also shown that the selective age-dependent decrease in MAO-B activity was due entirely to an increased enzyme concentration in brain tissue (Fowler et al. 1980b).

Student and Edwards (1977) demonstrated that MAO-B is predominantly localized in the neuroglia, a finding soon corroborated (Strolin Benedetti and Keane 1980) and now firmly established as fact.

In my hypothesis, put forth in 1981–1982, I suggested that a progressively developing biochemical lesion in the aging brain which leads to catecholaminergic and trace-aminergic deficiency is responsible for the age-related decline

in sexual and learning performances and ultimately leads to natural death (Knoll 1981a,b,c, 1982).

Let us quote here the original description of the hypothesis (Knoll 1982, pp. 109–110):

[W]ell established old experiences offer a good explanation for the increase of brain MAO-B activity in the latter decades of life. Cell loss is a general feature of the aging brain ... As the loss of neurons is always compensated by glial cells, the progressive and cumulative loss of neurons in the aging brain gives a satisfactory explanation to the selective increase of extrasynaptosomal MAO-B activity with increasing age. This seems to be an unavoidable biochemical lesion of aging ... Collating the facts that there is an unavoidable loss of neurons, inescapably leading to increased MAO-B activity with increasing age, makes it understandable that dopaminergic and trace-aminergic modulation in the brain is progressively decreasing in the aging brain. It is in agreement with this trend of changes that an age-dependent decrease in the dopamine control of the basal ganglia in man was described, first by Bertler (1961), and corroborated by many others. Riederer and Wuketich (1976) found that the dopamine content of the human caudate nucleus decreased in an age-related manner.

If, in addition, we also consider that the activity of tyrosine hydroxylase, the enzyme catalyzing the rate-limiting step in catecholamine biosynthesis, was also found to decrease in human brain tissue with increasing age (McGeer et al. 1971), weighty arguments seem to support the view that catecholaminergic tone is progressively decreasing in the aging brain.

As the described age-dependent chain of events can be deduced to well-defined biochemical lesions, the chances of developing a new drug strategy for counteracting or possibly even preventing the adverse consequences of the age-related decrease of the catecholaminergic tone in the brain are fair.

Dozens of studies published since the proposal of this hypothesis have strengthened this approach step-by-step. The evolution of the project can be followed via the reviews published after 1982, when the original hypothesis was presented, until the discovery of mesencephalic enhancer regulation (Knoll 1983, 1985, 1986a,b,c, 1989, 1992a,b, 1993a,b,c, 1995, 1998, 2001, 2003).

In the light of our present knowledge there can be little doubt that because of the continuously increasing MAO-B activity in the aging brain, the more and more efficient metabolism of PEA necessarily works against the chances of a freshly synthesized PEA molecule reaching its target. This is one of the factors which contributes to the age-related decline of mesencephalic enhancer regulation with the passing of time.

The same fits for dopamine. Figure 3.20 shows the decay in the dopamine content of the caudate nucleus in the aging human brain. We lose 13% of our brain dopamine in the decade after age 45. At this normal rate nobody will exceed, within the obtainable human life span, the critical threshold of dopamine content (30%) that accompanies the precipitation of the symptoms of Parkinson's disease. Thus, as illustrated in Fig. 3.20, Parkinson's disease is obviously

THE AGE-RELATED DECLINE OF STRIATAL DOPAMINE IN HUMANS

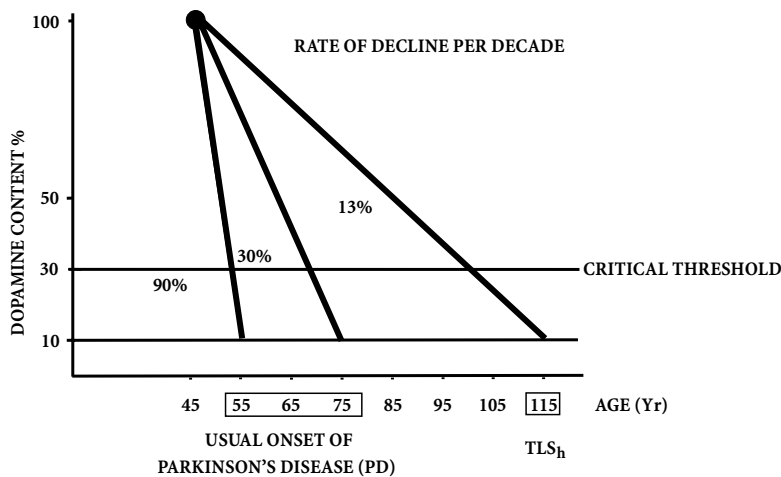


Fig. 3.20. Visualization of the concept that Parkinson’s disease (PD) is the premature rapid aging of the striatal dopaminergic machinery. TLS_h technical life span in humans

the consequence of a premature aging of the dopaminergic machinery in the human brain. To realize the functional consequences of the age-related decline of the dopaminergic system in the mesencephalon, let us follow, as an example, the decline of the ejaculatory activity in human and rat males 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.

Martin (1977) studied coital activity as a function of age, interviewing 628 members of the Baltimore Longitudinal Study of Aging. The subjects were white, married, urban residents in good health from the Washington-Baltimore area, varying from 20 to 95 years of age. According to this study, median coital activity was highest, 2.1 events/week, between the ages of 30 and 34, and decreased progressively with increasing age, sinking to 0.2/week in the age group 65–69.

The study throws light upon the enormous individual variation in sexual vigor. 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 1,000 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.

Table 3.4 summarizes the data of the human study to illustrate the age-related decline of coital activity and the striking individual differences in the sexual performance of human males in different age cohorts.

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. Table 3.5 shows the age-related decline of coital activity in male rats and the striking individual differences in copulatory activity in different age cohorts.

Because of brain aging, even the sexually high performing males necessarily 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 not later than at the completion of their second year of age (see Table 3.5).

We published a series of experiments in 1988 (see Table 3 in Knoll 1988), the results of which clearly show in retrospect that the age-related functional decline of the sexual performance of male rats can be taken as an indicator of the decay of mesencephalic enhancer regulation with the passing of time. We selected 132 sexually inexperienced 24-month-old rats for this experiment and tested their sexual activity in four consecutive weekly mating tests. Table 3.5 shows that none of them displayed ejaculation during the test period. Out of 132 animals, 46 did not show any sign of sexual activity, 42 displayed mountings only, and 44 displayed mountings and intromissions (sluggish rats).

Table 3.4. The age-related decline of coital activity in human males and percentages of striking individual differences from average copulatory activity in different age cohorts. Based on data from Martin (1977)

Age category	Age (years)	Average performance (sexual events/5 years)	Percentage of striking differences in performance from the average
Young adult males	20–39 (30–34)	~ 500 (~ 600)	~ 4% below 100 ~ 25% over 1,000
Mature adult males	40–49	300	~ 8% below 100 ~ 15% over 400 ~ 8% over 600
Aging and aged	65–80	Below 100	~ 3% over 400 ~ 1.5% over 600

Table 3.5. The age-related decline of coital activity in male CFY rats and the striking individual differences in the copulatory activity in different age cohorts

Age of rats (months)	Number of rats (n)	Complete inactivity n (%)	Mountings only n (%)	Mountings and intromissions n (%)	Full-scale activity (ejaculations) n (%)
3–6	381	21 (5.51)	20 (5.24)	140 (36.74)	200 (52.49)
12–18	138	27 (19.56)	27 (19.56)	76 (55.07)	8 (5.80)
24	132	46 (34.84)	42 (31.82)	44 (33.33)	0

Of the 132 rats, we treated half of the population (66 rats) with saline and we followed the sexual performance of the animals once a week until they died. The results shown in Table 3.6 allow the conclusion that the duration of the life of the rats was inversely proportional to their sexual performance. As sexual performance is directly proportional to the functional state of enhancer regulation in the dopaminergic neurons, we assume that the rat dies when the age-related decline in mesencephalic enhancer regulation arrives at a critical threshold. The finding that regarding sexual performance: Group 1 < Group 2 < Group 3 is just a sign that the 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 are the longest living.

The age-related decline in mesencephalic enhancer regulation during the postdevelopmental phase of life in male rats can further clearly be recognized by comparing the individual variation in sexual performance of 3- to 6-month-old male rats with the performance of their 2-year-old peers. Table 3.5 shows that whereas 52.49% of 3- to 6-month-old male rats displayed ejaculations during the four consecutive mating tests, only 5.80% of 12- to 18-month-old males ejaculated, and none of the 24-month-old males was in possession of this faculty any longer.

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

3.5.1.2.2

The Relative Weakness of the Brain Engine at the Time of an Emergency Incident (Natural Death): The Mechanism that Terminates Postdevelopmental Longevity

The rapid, age-related decline of the activity of the mesencephalic catecholaminergic system, which plays a key role in the activation of the cortex, suggested a reasonable explanation for the onset of natural death (see Knoll

Table 3.6. The dying out of 66 saline-treated male rats with differing sexual activities. Data taken from Knoll (1988)

Week of saline treatment	Number of dying animals in		
	Group 1 (noncopulators) (<i>n</i> = 23)	Group 2 (mountings only) (<i>n</i> = 21)	Group 3 (sluggish rats) (<i>n</i> = 22)
36 th	2		
37 th	4		
38 th	8	1	
39 th	-	-	
40 th	3	-	
41 st	5	1	
42 nd	1	7	
43 rd		6	1
44 th		3	4
45 th		-	1
46 th		2	2
47 th		1	5
48 th			3
49 th			1
50 th			2
51 st			-
52 nd			-
53 rd			-
54 th			-
55 th			-
56 th			2
57 th			-
58 th			-
59 th			-
60 th			1

1994, for review). According to this working hypothesis postdevelopmental longevity is terminated because the functional deterioration of the mesencephalic catecholaminergic machinery that activates the cortex sinks below a critical threshold in the aging brain. At the time of an emergency incident, when a high level of activation is needed to survive the tribulation, the cortical neurons cannot be further activated to reach the required level. Thus the essence of the natural death situation is the *relative* weakness of the brain engine. This would explain why a common infection, a broken leg, or any other challenge easily surmountable given catecholaminergic machinery

working at full capacity may cause death in old age. Continuous age-related decline of the brain engine's performance makes it unavoidable that with the passing of time every otherwise healthy living being arrives at natural death.

Due to inborn differences in the efficiency of the brain engine, we have in a random rat population extreme differences in performance. We can therefore create experimental circumstances in low-performing young rats leading to the *relative* weakness of the brain engine. We thus mimic a natural death situation to which otherwise healthy animals full of vigor fall victim.

In our effort to find for the rat the most favorable conditions for the acquisition of the glass-cylinder-seeking drive, we analyzed the kinetics of escape from a hot plate at different temperatures (see Chap. 3, pp. 43–53, in Knoll 1969, for review). The essence of the method (see Fig. 3 in Knoll 1969) was a copper hot plate (180 × 180 mm) the temperature of which could be regulated with an accuracy of $\pm 0.2^\circ\text{C}$. The metal surface was heated to the desired temperature and a glass cylinder, 30 cm high, with bottom and top diameters of 16 and 12 cm, was placed at the top of it. The glass cylinder, opened on bottom and top, had no side opening. The experiment was performed on female rats weighing 120–150 g. The animals were dropped onto the heated metal plate, through the upper opening of the glass cylinder. The time elapsing between their fall and subsequent jump onto the cylinder's top was measured. Ten such tests were usually performed at 30 s intervals. If the animal had not jumped out of the glass cylinder in 4 h in any of the tests, no further measurements were made and the 4 h time was referred to as the maximum value.

Although the experimental circumstances are peculiar, in essence we activate an inner drive. The animal fights for survival and mobilizes all resources to find the way of escape by trial and error. What definitely happens in the brain is that the heat/pain stimulation activates the brain engine and in proportion with it the cortical neurons start working on a high-activity level, allowing the acquisition of those chains of ECRs that ultimately enables the rats to find, by trial and error, the way to escape from the hot plate. Rats with inadequately activated brain engines are in danger.

At 40°C , the animals stayed in the glass cylinder throughout the whole period of 240 min, thus, this stimulus was subliminal to properly activate the brain engine and cortical neurons toward eliciting the escape reaction. A temperature of 45°C was the lowest that elicited jumping onto the rim of the upper opening of the glass cylinder. The time needed for escape at this temperature varied between 25 and 164 min in a group of naive rats ($n = 10$) (see Table 13 in Knoll 1969). These conditions were harmless and the experiment did not change the health of any of the rats.

Raising the temperature of the hot plate to 50°C changed the situation significantly. This not too intensive heat/pain stimulus is insidious and becomes

life threatening for a low-performing rat. At this temperature six out of a group of ten naive rats escaped within 2–13 min and remained healthy. Four rats, however, were unable to cope with the situation; three of them died and the fourth became seriously ill. Table 3.7 shows the corresponding details.

Heating the hot plate to 55 °C yielded a more favorable result. Nine out of a group of ten rats escaped between 1 and 6 min and remained healthy. One animal needed 14 min to escape and later became seriously ill (see Table 15 in Knoll 1969).

The heat/pain stimulation via a hot plate at 60 °C confused some of the animals and, out of a group of ten, three died and one became ill (see Table 16 in Knoll 1969). Nevertheless, for producing glass-cylinder-seeking rats we used this temperature as it proved to be the most efficient.

The success of the escape of naive rats from the hot plate depends primarily on the efficiency of their mesencephalic catecholaminergic machinery. This was shown in rats pretreated for 4 weeks, once daily, with 0.1 mg/kg reserpine. None of a group of ten reserpine-treated rats escaped from a hot plate heated to 55 °C; they all died. However, seven out of ten reserpine-treated rats safely escaped when the temperature of the hot plate was raised to 60 °C (see Table 19 in Knoll 1969).

Altogether, the data substantially support the view that natural death sets in when the catecholaminergic system's ability to activate the telencephalon sinks, for whatever reason, below a critical threshold, and during a state of emergency when a high level of activation is needed to survive the tribulation, the cortical neurons cannot reach the necessary level of activation.

The results shown in Table 3.7 are especially apt in support of this conclusion. There are innate differences in the efficiency of the mesencephalic catecholaminergic system. For the brain of animals (nos.) 1, 2, 4, 5, and 6 the hot plate heated to 50 °C was already an adequate stimulus to enhance the excitability of the cortical neurons for escape and to survive the tribulation without perceivable consequences. Animals 3 and 7 escaped, but fell ill thereafter. Animals 8, 9, and 10 were unable to cope with the situation and died.

3.5.2

Rationale for Slowing the Age-Related Decline of Mesencephalic Enhancer Regulation by the Daily Administration of a Small Dose of a Synthetic Mesencephalic Enhancer Substance from Sexual Maturity Until Death

In light of the peculiar changes in enhancer regulation during the developmental phase of life, the antiaging potential of the administration of a small dose of a safe enhancer substance during the postdevelopmental (aging) phase of life deserves serious consideration. It seems reasonable to shift safely the functional constellation of the brain during postdevelopmental longevity to-

Table 3.7. Escape of rats from a hot plate at 50 °C. Optimal temperature to mimic a “natural death” situation for low-performing rats

Serial no.	Jumping reaction times in rounded-off minutes on experiment day										Note
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	
1	2	1	2	1	1	1	1	1	1	1	
2	2	1	2	2	2	1	1	1	1	1	
3	34	1	2	3	12	11	3	3	1	1	ill
4	10	1	1	4	7	1	1	1	1	1	
5	13	7	4	8	5	7	2	1	1	1	
6	4	1	3	3	13	1	1	1	1	1	
7	8	7	4	2	2	8	2	1	2	2	ill
8	34	14	32	2	22	6	6	2	-	-	ill, died
9	49	38	-	-	-	-	-	-	-	-	ill, died
10	63	-	-	-	-	-	-	-	-	-	ill, died
Mean	22	8	6	3	8	5	2	1	1	1	

Mean rectal temperature on day 1: immediately after 1st measurement = 38.8 ± 0.9 °C, immediately after 10th measurement = 39.7 ± 1.0 °C, 30 min after 10th measurement = 37.1 ± 0.4 °C
See Chap. 3 in Knoll (1969) for methodological details

wards this one characteristic of the uphill period of life (from weaning until sexual maturity).

To keep the engine of their brain on a higher activity level during post-developmental longevity, humans need lifelong medication. It is reasonable to start with the daily administration of a small amount of a synthetic mesencephalic enhancer substance immediately following sexual maturity. This will work for decades. It will improve the quality of life in the latter decades, increase the chances for a longer life, and decrease the danger of precipitating age-related depression and neurodegenerative diseases (Parkinson's, Alzheimer's).

As to the mechanism of the antiaging effect of synthetic mesencephalic enhancer substances, we need to recapitulate the essence of the enhancer effect as was shown in Figs. 3.1 and 3.2. Due to enhanced excitability, a higher number of the enhancer-sensitive neurons in the brain stem get activated to a given stimulus under the influence of natural enhancer substances, PEA and tryptamine. Synthetic mesencephalic enhancer substances, (–)-deprenyl and (–)-BPAP, act similarly (see Sect. 3.1.3). Due to the age-related continuous, slow decline of mesencephalic enhancer regulation during postdevelopmental longevity, the number of the enhancer-sensitive mesencephalic neurons which are activated in response to a given stimulus decreases proportionally over time. Since synthetic mesencephalic enhancer substances increase the excitability of the enhancer-sensitive mesencephalic neurons, it can reasonably be expected that their proper administration during the postdevelopmental phase of life will slow the age-related decay of mesencephalic enhancer regulation. In this sense a synthetic mesencephalic enhancer substance is an antiaging drug.

As an example, the functional decline of the mesencephalic dopaminergic neurons with the passing of time is convincingly demonstrated both in human and rat males in Tables 3.4 and 3.5. The conspicuous age-related decay of sexual performance is the proof of the decline of enhancer regulation in the mesencephalic dopaminergic neurons in aging human and rat males. The same stimulus that activated a high number of the dopaminergic neurons in the young males will activate lower and lower number of these neurons as time passes. Since the administration of a synthetic mesencephalic enhancer substance will increase, by its specific effect, the excitability of the dopaminergic neurons, treatment of an aged male with such a compound can be expected to enhance sexual activity and bring it back to a level that was characteristic of an earlier stage of life of the individual. We have proved experimentally in a series of studies the validity of this assumption.

To date longevity studies have been performed only with (–)-deprenyl, as this compound was the only available synthetic mesencephalic enhancer substance. To illustrate the antiaging effect of (–)-deprenyl, Table 3.8 shows some data taken from our first longevity study on male rats performed between 1984 and 1988. We started the experiments with 2-year-old male rats. Before

Table 3.8. Antiaging effect of (–)-deprenyl treatment. Data taken from Table 4 in Knoll (1989). Details explained in text

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
<i>Saline-treated rats</i>				
Non-copulators	23	37	0	0
Mounting rats	21	425	54	0
Sluggish rats	22	477	231	0
<i>(-)-Deprenyl-treated rats</i>				
Non-copulators	23	997	544	190
Mounting rats	21	1,129	662	172
Sluggish rats	22	1,696	1,257	481

treatment the sexual performance of the rats was measured in four consecutive weekly mating tests. Three of the characteristic patterns of sexual behavior, mounting (M), intromission (I), and ejaculation (E) were monitored. Rats were classified as “noncopulators” (no sign of sexual activity), “mounting rats” (displayed mountings only), and “sluggish rats” (displayed mountings and intromissions) (Knoll et al. 1983; Dalló et al. 1986a,b; Knoll et al. 1989).

In the four mating tests performed before treatment, out of the 132 males selected for this experiment, 46 were found to be noncopulators; 42 animals displayed mountings only, and 44 rats proved to be sluggish. None of the rats displayed ejaculation. It was our intention to start the experiment with 2-year-old rats as we knew from our earlier studies that the strain of CFY rats we used in our experiments lost the ability to ejaculate before they completed their 2nd year of age.

After classifying our male rats according to their sexual performance in the testing period, we started treating rats s.c. with saline (1 ml/kg) and (–)-deprenyl (0.25 mg/kg), respectively, three times a week, until they died. We tested their sexual performance once a week. In each group half of the animals were treated with saline and half with (–)-deprenyl.

Table 3.8 shows the total number of mountings, intromissions, and ejaculations displayed by the whole group of rats until they died. The noncopulator group of the saline-treated rats displayed only 37 mountings altogether. In harmony with the results of the four initial mating tests, these rats were really sexually inactive. In the saline-treated group of rats that displayed mountings only in the initial test, the total number of mountings (425) was much higher than in the noncopulator group, and altogether 54 intromissions were produced. The saline-treated sluggish rats displayed 251 intromis-

sions. As expected, no single ejaculation was detected in the 66 saline-treated males.

In the (-)-deprenyl-treated group the total number of mountings and intromissions increased tremendously and ejaculations were also displayed. Thus due to the enhancer effect of (-)-deprenyl the excitability of the mesencephalic dopaminergic neurons was increased and the efficiency of sexual performance of the rats changed accordingly.

To further illustrate the peculiar antiaging effect of a synthetic mesencephalic enhancer substance, the results of characteristic human and rat studies are summarized in Figs. 3.21 and 3.22, respectively.

Figure 3.21 illustrates the findings of the DATATOP study performed on selected patients with early, untreated Parkinson's disease (Parkinson Study Group 1989). The 401 placebo-treated patients (P) needed levodopa significantly earlier than the 399 (-)-deprenyl-treated patients. The reason for the observed effect is clear. The diagnosis of Parkinson's disease indicates that the decrease in the dopamine content of the caudate nucleus had already exceeded the critical threshold (30%). Because the dopaminergic system continues to deteriorate, the next precisely measurable step of decay is the point at which the patient requires levodopa. Fig. 3.21 shows that

Fig.3.21. The rate of deterioration from diagnosis to the need for levodopa in parkinsonian patients treated with placebo (P) and (-)-deprenyl (D), respectively. Based on the data published by the Parkinson Study Group (1989)

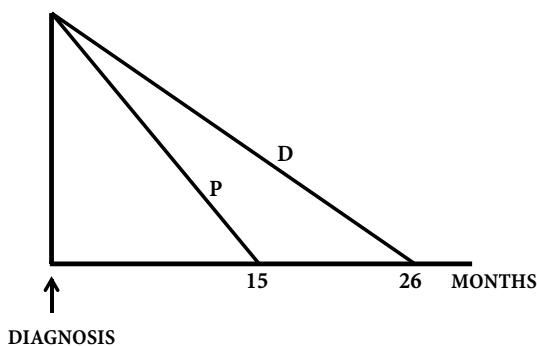
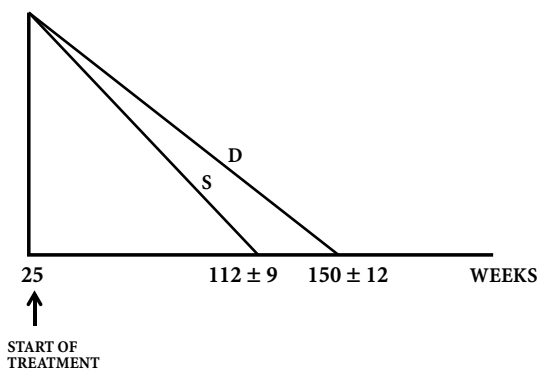


Fig.3.22. The age-related decline of the sexual potency of male CFY rats treated with saline (S) and (-)-deprenyl (D), respectively, until they lost the ability to ejaculate



the enhancer effect of (–)-deprenyl significantly delayed the onset of this stage.

Figure 3.22 shows essentially the same effect of (–)-deprenyl on male CFY rats. The experiment was performed on 90 selected young males possessing full-scale sexual activity. Half of the population was treated with saline (1 ml/kg), the other half with (–)-deprenyl (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 of 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, (–)-deprenyl-treated rats reached it at an average of 150 ± 12 weeks ($P < 0.001$) (Knoll 1993a). As sexual performance is a dopaminergic function, it is obvious that the enhanced activity of the mesencephalic dopaminergic neurons was responsible for the significantly retarded loss of the ability to ejaculate in the (–)-deprenyl-treated group.

*

Due to its enhancer effect (–)-deprenyl protects the nigrostriatal dopaminergic neurons against selectively acting neurotoxins (Knoll 1978; Hársing et al. 1979; Cohen et al. 1984; Finnegan et al. 1990; Vizuete et al. 1993; Wu et al. 1993), and facilitates scavenger function in the striatum (Knoll 1988, 1990; Carrillo et al. 1991, 1992).

In a series of experiments we also succeeded in finding morphological evidence that treatment of rats with a synthetic mesencephalic enhancer substance slows aging of the neurocytes of the substantia nigra of rats. We developed a method, using a TV-image analyzer, to compare different morphological parameters in the substantia nigra of young and old male rats (Tóth et al. 1992). With the aid of this method, we determined the number, total area, area of one granule, and density features (sum and average of gray values and average gray value of one pigment granule) of melanin granules in neurocytes of the substantia nigra in 3-month-old and 3-year-old male rats (Knoll et al. 1992b). The number of cells in sections of identical areas was similar in the young and old rats. A statistically nonsignificant difference between the two age cohorts in the proportion of neurocytes with and without melanin was found: 773 (48.1%) with and 853 (51.8%) without in the young rats and 1,219 (65.1%) and 652 (34.8%) in the old ones. Within the melanin-containing neurocytes, however, statistically significant age-related differences in the number, area, and density features of melanin granules were discovered. The majority of the neurocytes in young rats contained numerous, small-sized neuromelanin granules, whereas in the majority of the neurocytes of old rats, smaller numbers of large-sized neuromelanin granules were detected.

Table 3.9 shows examples of the characteristic, highly significant differences in the morphological fine structure of the neuromelanin granules in the neurocytes of the substantia nigra of 3-month-old and 3-year-old rats.

The statistically significant age-related differences revealed by the TV-image analyzer allowed us to check morphologically the influence of long-term (–)-deprenyl treatment on the neurocytes of the substantia nigra.

Table 3.10 shows that (–)-deprenyl, injected subcutaneously three times a week for 18 months, prevented the age-related morphological changes of the pigment granules in the neurocytes of the substantia nigra of rats. This is morphological evidence for the antiaging effect of prophylactic treatment with a synthetic mesencephalic enhancer substance.

Rinne et al. (1991) found that the number of medial nigral neurons was greater and the number of Lewy bodies fewer in those parkinsonians who had been treated with (–)-deprenyl in combination with levodopa when compared with patients who had received levodopa alone.

Table 3.9. Age-related differences in the number of neuromelanin granules and in the area of one granule in the neurocytes of the substantia nigra. Data taken from Table 3 in Knoll (1992b)

	Neurocytes from				<i>P</i>
	3-month-old rats (<i>n</i> = 773)		3-year-old rats (<i>n</i> = 652)		
	Mean	SD	Mean	SD	
Number of neuromelanin granules in a neurocyte	2,600	1,500	1,800	900	< 0.0001
Area of one granule (μm ²)	49,000	18,800	116,300	29,500	< 0.0001

Table 3.10. Age-related changes in the number of neuromelanin granules and in the area of one granule in the neurocytes of the substantia nigra and prevention of these changes by (–)-deprenyl treatment (0.25 mg/kg, s.c. three times a week for 18 months). Data taken from Table 3.3 in Knoll (1992b)

	3-month-old rats (YC) (<i>n</i> = 473)		Neurocytes from 21-month-old rats treated for 18 months with saline (OC) (<i>n</i> = 481)		21-month-old rats treated for 18 months with (–)-deprenyl (DT) (<i>n</i> = 503)	
	Mean	SD	Mean	SD	Mean	SD
(1) Number of neuromelanin granules in a neurocyte	2,000	1,100	1,700	920	2,000	1,100
(2) Area of one granule (μm ²)	39,600	25,000	75,000	30,600	39,800	19,600

(1) YC vs OC *P* < 0.002; YC vs DT *P* > 0.05

(2) YC vs OC *P* < 0.0001; YC vs DT *P* > 0.05

3.5.3

Clinical Experiences with (–)-Deprenyl in Depression and in Neurodegenerative Diseases: Further Therapeutic Prospects

Depression. The logic indication for the use of synthetic mesencephalic enhancer substances is their prophylactic administration to slow the physiological age-related decay of mesencephalic enhancer regulation. Nevertheless, as discussed already earlier (see Sect. 3.1.2), (–)-deprenyl, the PEA-derived synthetic mesencephalic enhancer substance, was originally developed with the intention to use it as a new spectrum antidepressant.

The antidepressant effect of the compound was first demonstrated by Varga (1965) and Varga et al. (1967) with the racemic form, and in 1971 with the (–) enantiomer (Tringer et al. 1971). The first study that corroborated the antidepressant effect of (–)-deprenyl was published by Mann and Gershon (1980).

The realization of the peculiar effect of (–)-deprenyl, first in Parkinson's disease and later in Alzheimer's disease, distracted attention from its antidepressant property, which remained unutilized. Even an especially interesting aspect of this problem fell into oblivion. In a study performed by Birkmayer et al. (1984) on 102 outpatients and 53 inpatients, (–)-deprenyl was given together with (–)-phenylalanine. The latter is a precursor of PEA that, in contrast to PEA, crosses the blood-brain barrier and, as it is metabolized in the brain, increases the concentration of this natural enhancer substance. Nearly 70% of the patients achieved full remission. This outstanding clinical efficiency was equaled only by that of electroconvulsive treatment, but without the latter's side effect of memory loss.

As the drug was primarily used in Parkinson's disease and this illness is very often accompanied by depression, clinicians who treated Parkinson's disease with (–)-deprenyl realized and described the antidepressant effect of the drug in parkinsonism (Youdim 1980; Tom and Cummings 1998; Miyoshi 2001; Zesiewicz et al., 1999).

Quitkin et al. (1984) found (–)-deprenyl effective against atypical depression. This open trial on 17 patients made the finding questionable. But McGrath et al. (1989) in a placebo-controlled trial of (–)-deprenyl proved the efficiency of the drug in atypical depression. In a double blind evaluation Mendlewicz and Youdim (1983) found (–)-deprenyl treatment successful in major depression. Some authors (Lees 1991; Kuhn and Muller 1996; Ritter and Alexander 1997) realized marked antidepressant effect of high doses of (–)-deprenyl (40–60 mg/day). In a study by Bodkin and Amsterdam (2002) the transdermal application of (–)-deprenyl was effective in double-blind, placebo controlled, parallel group examinations in depressed outpatients. Amsterdam (2003) checked the trial and found a good antidepressant effect of (–)-deprenyl. Considering the results of the detailed pharmacological analyses with (–)-deprenyl and with (–)-PPAP, its close structural analogue free

of MAO-B inhibitory potency, there is good reason to accept the conclusion that the observed antidepressant efficacy of the drug is unrelated to MAO-B inhibition and that the enhancer effect of the compound is fully responsible for this effect (Knoll 1998).

(-)-BPAP is about 130 times more potent than (-)-deprenyl in rats for antagonizing tetrabenazine-induced depression in the shuttle box. As was discussed previously (Sect. 3.1.3.2), (-)-BPAP, in contrast to (-)-deprenyl, is a highly efficient enhancer of the serotonergic neurons in the mesencephalon. In all the experimental studies performed with (-)-BPAP in comparison to (-)-deprenyl, the tryptamine-derived enhancer substance proved to be substantially more potent. There is good reason to expect that (-)-BPAP will in all likelihood significantly surpass the antidepressant effect of (-)-deprenyl. As is well-known, a considerable percentage of sufferers of major depression cannot be cured with the available antidepressants. Since selective uptake inhibitors, the most effective antidepressants presently used, and enhancer substances stimulate the catecholaminergic and serotonergic neurons in the brain via quite different mechanisms (Miklya and Knoll 2003), their combination with synthetic mesencephalic enhancer substances opens up a new prospect for treating depressed patients in the future.

Parkinson's Disease. The neostriatum is the main input structure of the basal ganglia. We have to consider the physiological role of the nigrostriatal dopaminergic neurons in the continuous activation of the cerebral cortex (see Mink and Thach 1993; Standaert and Young 1996, for review). This is realized via a highly complicated route of connections (see Albin et al. 1989, for review). The neostriatum is the main input structure of the basal ganglia. It gets 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 is increasing 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 D₁ dopamine receptors; the striatal neurons of the indirect pathway express primarily the inhibitory D₂ 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*, the physiological age-related decline of the nigrostriatal dopaminergic activity leads to an equivalent reduction in the activity of the cerebral cortex. It is reasonable to conclude that the age-related decline of the nigrostriatal dopaminergic brain mechanism plays a significant role in the decline of performances over time.

Aging of the dopaminergic system in the brain plays an indisputably 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. As discussed above (see Sect. 3.5.1.2.1) in a human male study median coital activity was the highest, 2.1 events/week, between the ages of 30 and 34, and decreased progressively with increasing age, sinking to 0.2/week ($P < 0.001$) in the 65- to 69-year-old age group. We found essentially the same trend of changes in male rats in a different series of experiments.

There is a quantitative difference only between the physiological age-related decline of the dopaminergic input and that observed in Parkinson's disease. 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 Parkinson's disease is 70% or thereabout at diagnosis and over 90% at death. The drastic reduction of the dopaminergic output in Parkinson's disease evidently leads to an accordingly drastic reduction of cortical activity and this makes it clear why an enhancer substance, like (–)-deprenyl, improves cognition, attention, memory and reaction times and brings about subjective feelings of increased vitality, euphoria and increased energy in people with Parkinson's disease (see Sect. 3.2.1).

In diagnosing Parkinson's disease, the neurologist selects subjects with the most rapidly aging striatal dopaminergic system (about 0.1% of the population). As symptoms of Parkinson's disease 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. Prevention is the only chance to fight off Parkinson's disease. We need to start slowing the age-related functional decline of mesencephalic enhancer regulation in due time. For this reason it is advisable to begin the prophylactic administration of a synthetic mesencephalic enhancer substance, for example 1 mg (–)-deprenyl/day, as soon as sexual maturity has been reached and the postdevelopmental period of life has just started. It is therefore of particular importance that (–)-deprenyl, to date the only synthetic mesencephalic enhancer substance in clinical use, has proven to be an unusually safe drug.

The DATATOP study in the USA (Parkinson Study Group 1989), the French Selegiline Multicenter Trial (FSMP) (Allain et al. 1991), the Finnish study (Myttilä et al. 1992), and the Norwegian-Danish Study Group (Larsen et al. 1999), all multicenter studies that used (–)-deprenyl as initial treatment in *de novo* patients with Parkinson's disease, have supported the conclusion that (–)-deprenyl slows the progression of early Parkinson's disease and have demonstrated the safety of the long-term administration of the drug. It is commonly assumed that (–)-deprenyl by itself is an exceptionally safe compound.

Due to the inhibition of MAO-B, (–)-deprenyl treatment allows for a 20–50% decrease in the levodopa dose needed in Parkinson's disease. In patients who need levodopa, however, there is always a risk that the administration of (–)-deprenyl 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. An example of a multicenter clinical trial in which the improper combination of levodopa with (–)-deprenyl led to confusion and misinterpretation is the one performed by the Parkinson's Disease Research Group of the United Kingdom (PDRG-UK) (Lees 1995).

Quite unexpectedly, this group published an alarming paper claiming that parkinsonian patients treated with levodopa combined with (–)-deprenyl show an increased mortality in comparison with the patients treated with levodopa alone (Lees 1995). This finding was in striking contradistinction to all other studies published in a variety of countries. Birkmayer et al. (1985) even found an increased life expectancy resulting from the addition of (–)-deprenyl to levodopa treatment in Parkinson's disease. The "idiosyncratic prescribing" (Dobbs et al. 1996) of (–)-deprenyl in combination with levodopa in the PDRG-UK study led to the false conclusion of the authors. Comments (Dobbs et al. 1996; Knoll 1996; Olanow et al. 1996) pointed uniformly to the substantial overdosing of levodopa as the cause of the observed deaths with (–)-deprenyl as an adjuvant in this trial.

There are several promising opportunities in the treatment of Parkinson's disease: 1. to slow the progress of the disease and delay the timepoint at which levodopa is needed in *de novo* patients with Parkinson's disease by administering (–)-BPAP, a much more potent and selective enhancer substance than (–)-deprenyl; 2. to administer a carefully adjusted dose of (–)-deprenyl when levodopa is already needed; and 3. to make a safe use of the levodopa-sparing effect of (–)-deprenyl.

Since the diagnosis of Parkinson's disease is the unequivocal proof that the age-related irreversible deterioration of the nigrostriatal dopaminergic neuronal system has already surpassed a critical level in the patient, and the disease is incurable, prevention remains the only chance for the future to fight off Parkinson's disease. The daily administration of a small dose of a synthetic mesencephalic enhancer substance from sexual maturity until death presents itself as a proper method for reaching this aim.

Alzheimer's Disease. Alois Alzheimer described in 1907 the form of dementia that bears his name. He was the first who pointed to a relationship between dementia and the extensive appearance of dense fiber-like tangles and darkly staining senile plaques in the cortical and hippocampal regions. The grave morphological changes lead to grave functional disturbances. For example, the loss of pyramidal neurons and their synapses necessarily leads to cholinergic and glutamatergic hypofunction. As the important role of these transmissions in cognitive and memory functions is well-known, the current symptomatic treatment of Alzheimer's disease is based on the correction of these hypofunctions. But none of these strategies came up to expectations.

Another strategy to treat Alzheimer's disease, the "β-amyloid cascade" theory, is based on neuropathological changes verified postmortem, the excessive appearance of which is claimed to be characteristic of the disease: accumulation of neurofibrillary tangles composed of hyperphosphorylated tau proteins and extracellular senile plaques containing β-amyloid₁₋₄₀ and β-amyloid₁₋₄₂ (see Morishima-Kawashima and Iharra 2002, for review).

As β-amyloid₁₋₄₂ is a neurotoxic agent, the hypothesis that this is a key molecule in the pathology of Alzheimer's disease is now widely accepted (see Selkoe 2001, for review). The theory is controversial since the correlation between the concentrations and distribution of amyloid depositions in the brain and parameters of Alzheimer's disease pathology, such as the degree of dementia, loss of synapses, and loss of neurons, is poor (Neve and Robakis 1998).

As neurotoxicity is thought to be inseparable from oxidative injuries, free radicals, calcium and inflammatory-mediated processes, agents with protective effect on cultured neurons, anti-oxidant compounds, and anti-inflammatory drugs are continuously tested in Alzheimer's disease. For example, vitamin E and selegiline (Sano et al. 1997; Grundman 2000; Thomas 2000; Kitani et al. 2002; Birks and Flicker 2003), Ginkgo biloba extract (Ponto and Schultz 2003), nonsteroidal anti-inflammatory drugs (Etminan 2003), estrogen (Schumacher et al. 2003) have been administered.

Despite the rapid growth of the number of papers that bear evidence of the seemingly frantic success of the "β-amyloid cascade" theory, a survey of the literature furnishes unequivocal evidence that the therapy based on this strategy has not changed the hopelessness of the patients who had already developed Alzheimer's disease. A radically new approach is needed to curb the predicted dramatic increase in the prevalence of Alzheimer's disease.

The first two studies to demonstrate the beneficial effect of (–)-deprenyl in Alzheimer's disease were published in 1987 (Martini et al. 1987; Tariot et al. 1987), and a series of clinical studies with small sample sizes confirmed thereafter the usefulness of this drug in the treatment of the disease (see Knoll 2001, for review). In some of these studies the effect of (–)-deprenyl was compared with other drugs. Campi et al. (1990) found (–)-deprenyl to be

more effective than acetyl-L-carnitine. According to Falsaperla et al. (1990), (–)-deprenyl is more effective than oxiracetam (a piracetam-like nootropic drug) in improving higher cognitive functions and reducing impairment of daily living. In a study by Monteverde et al. (1990) (–)-deprenyl proved to be more effective than phosphatidylserine.

The rationale and design of the first multicenter study of (–)-deprenyl in the treatment of Alzheimer's disease using novel clinical outcomes was published by Sano et al. in 1996 and the results of this study were published 1 year later (Sano et al. 1997). The primary outcome involved the time that elapses until the occurrence of any of the following: death, institutionalization, loss of the ability to perform basic activities of daily living, or severe dementia. There were significant delays in the time taken for such primary outcomes to occur in patients treated with (–)-deprenyl. The authors concluded that in patients with moderately severe impairment from Alzheimer's disease, treatment with (–)-deprenyl slows the progression of the disease.

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The operation of mesencephalic enhancer regulation provides a true perspective on the development of the two main neurodegenerative diseases (Parkinson's, Alzheimer's). As has already been discussed above in connection with Parkinson's disease, a more active brain engine means a more active cerebral cortex. Accordingly, the mesencephalic brain engine keeps the telencephalic neurons continuously active in the developmental phase of life and in the early phase of postdevelopmental longevity. These neurons fight successfully against their innate self-produced harmful metabolites. Due to the irresistible, slow decay of mesencephalic enhancer regulation as a function of age, the beneficial influence of the brain engine on the telencephalic neurons progressively declines. As a result of this process, with the passing of time even morphologically traceable age-related neurodegenerative changes appear in the highly sensitive, most sophisticated telencephalic neurons.

Alzheimer's disease – an irreversible loss of neurons primarily in the cortex and hippocampus leading to progressive impairment in memory, judgement, decision making, and so on – is the worst outward form of brain aging. An analysis of the prevalence of Alzheimer's disease as a function of age makes it clear that this is just a grave form of the natural aging of the human brain.

The mean age at the onset of Alzheimer's disease is approximately 80 years, and the manifestation of the illness before the age of 60–65 years is very rare. In the age cohort 65–69, Alzheimer's disease has a prevalence of only 1%. This increases to about 20% in the 85- to 89-year-old group and the risk of precipitating the disease can reach the 50% level among persons 95 years of age and over (Campion et al. 1999; Hy et al. 2000; Helmer et al. 2001; Nussbaum and Ellis 2003). The prevalence of Parkinson's disease over the age of 80 is only 1–3% (Tanner et al. 1996).

In the population over 65, there are substantial sex (68% female, 32% male) and geographical (2.1% Japan, 5.2% Europe and 10% USA) differences in the incidence of Alzheimer's disease (see Lockhart and Lestage 2003, for review). The disease now affects about 15 million persons worldwide, and a sharp increase in the afflicted population is expected in the future as it is estimated that the number of individuals over 65 will increase to 1.1 billion by 2050. It is therefore a pressing and no longer postponable necessity to find a safe and efficient prophylactic therapy to significantly decrease the prevalence of Alzheimer's disease as soon as possible.

There are special genetic risk factors for Alzheimer's disease – such as, for instance, the $\epsilon 4$ allele of the apolipoprotein E (APOE) gene, isotonic variation in CYP46, CYP46*C – that significantly increase the risk of Alzheimer's disease development (Bookheimer et al. 2000; Borroni et al. 2004). Accordingly, it seems reasonable to assume that the majority of those who precipitate the disease are carriers of risk factors.

The only reasonable hope to fight off Alzheimer's disease in the future is to keep the cortical and hippocampal neurons at a higher activity level as long as possible by the prophylactic administration of a synthetic mesencephalic enhancer substance. It is remarkable in this regard that (–)-BPAP protected cultured rat hippocampal neurons from the deleterious effect of β -amyloid_{25–35} fragments in as low as 10^{-15} M concentration (Knoll et al. 1999).

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