

Nerve Growth Factor Differentially Affects Spatial and Recognition Memory in Aged Rats

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Abstract In rats, object discrimination depends on the integrity of the cholinergic system, thus it could be expected that nerve growth factor (NGF) can improve the behavior in aged subjects. The interactive effect of age and cholinergic improvement was assessed behaviorally in young and aged rats. Animals were injected by infusion of NGF into the lateral ventricles and they were tested in two behavioral tasks: an object-location and an object-recognition task. Spatial and recognition memory were assessed in an open field containing five different objects. Rats were submitted to six consecutive sessions. Both age-groups showed comparable habituation of exploratory response in Session 1–4. Discrimination index (DI) was calculated to assess responses to spatial change in Session 5 and object change in Session 6. Control young and aged rats were able to discriminate between familiar and novel object, however DI was lower in aged rats. Treatment with NGF induced decline of object discrimination in both age-groups. Different results were obtained in spatial displacement test. NGF was able to improve spatial memory in aged rats, but had no effect in young controls. These data confer on NGF potential role in improving spatial but not episodic memory in aged rats.

Keywords Aging · Cholinergic system · Neurotrophins · Memory · Rats

Introduction

Brain aging is characterized by cognitive deficits and associated with morphological and biochemical changes, not the least of which are substantial changes in central cholinergic system [1–3]. The age associated impairment of the cholinergic system is demonstrated by: decrease of acetylcholine release from the cerebral cortex, as shown in rodents [4–6] and humans [7], by a decrease in number and change in size of cholinergic neurons [8–10], by a reduction in cholinergic activity in several brain areas [11, 12], and by changes in cholinergic receptor density and affinity [13–15]. A perturbation of cholinergic innervation is likely to be present even in the very early stages of Alzheimer's disease (AD). A substantial loss of cholinergic innervation in the cerebral cortex is universally accepted as a major aspect of advanced AD [16–18].

The correction of the cholinergic hypofunction has been so far the main aim of the pharmacological treatment of AD and a large number of drugs have been proposed. Among them, the compounds on which the largest number of pre-clinical and clinical investigations has been carried out are the cholinesterase inhibitors [19]. However, the cholinergic inhibition therapies are unlikely to offer definitive treatment for AD. They are likely to remain major component of a concerted approach aiming to influence the onset and progression of neurodegeneration from as many directions as possible. Proper function of basal forebrain (BF) cholinergic neurons depends on a continuous

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supply of target-derived trophic factors such as the nerve growth factor (NGF) [20, 21]. It is assumed that, by stimulating the activity of the BF cholinergic neurons, NGF might also restore the cholinergic transmission in the brain. NGF is known to promote the survival, sprouting, and phenotypic expression of these neurons to various degrees in developing and mature organisms [22–25]. Although target-derived NGF does not regulate the survival of mature cholinergic neurons, existent evidence suggests that the production of transmitter-synthesizing enzyme ChAT, cell size, and terminal arborization of these neurons is regulated by NGF [23]. The age-related reduction in NGF accessibility probably contributes to the pronounced vulnerability of cholinergic neurons to degeneration during aging [26, 27] and in AD [28–30].

A number of *in vivo* studies have demonstrated that age-related dysfunction of cholinergic system might be ameliorated by treatment with NGF [10]. Treatment with exogenous NGF prevents the memory loss and degeneration of cholinergic neurons associated with aging in rats [31–33] and in primates [34, 35]. In view of these observations, NGF has been proposed as a possible therapeutic agent in neurodegenerative disorders involving cholinergic neuronal atrophy such as AD. However, the question whether there is a relationship between cholinergic and memory decline is still a matter of investigation [36]. In young adult rodents, the selective immunolesioning of cholinergic neurons in the BF causes learning and memory impairments in some experiments, but not in all [37–39]. Similarly in primates, even non-selective destructive lesions that include the cholinergic as well as non-cholinergic components of the nucleus basalis have lead to memory deficits in some experiments [40, 41], but not in others [42]. With regard to aging, there are numerous publications showing that aged rats are impaired on various aspects of cognition, such as spatial learning [43–46], short-term memory [47, 48], and attentional processes [49, 50]. While these memory deficits are generally considered as reflecting age-related decline in cognitive capabilities, the results obtained from studying the cholinergic system in laboratory animals during normal aging appear to be contradictory [51, 52]. Even in experiments in which learning deficits emerge after cholinergic denervation, the deficits have been attributed to attentional rather than mnemonic factors [53, 54]. Current views of BF function suggest a selective role for BF cholinergic neurons in the modulation of attention [55–57].

The aim of our study was to investigate whether and to what extent spatial and recognition memory is impaired by age and cholinergic hypofunction and

whether the potential impairment can be corrected by NGF infusions. In order to assess this, cognitive behavior of young and aged rats were examined in object-location (OLT) and object-recognition test (ORT) in open field (OF) and after habituation, their response to displaced and novel object was assessed. In the following experiment, animals were confronted with several simultaneously presented objects and must respond to relevant one while disregarding the irrelevant others [58]. As might be expected on the basis of relevant literature, learning performance in this test is sensitive to the effect of aging and cholinergic dysfunction [59, 60] and depends, at least partly, on selective attention capability [61, 62].

Experimental procedure

Subjects

Twenty-four male Wistar rats (Animal Farm of the Nofer Institute of Occupational Medicine, Lodz, Poland) of 4 ($N = 13$) and 28 ($N = 11$) months of age were used. The rats were housed in groups of four in macrolon cages with *ad libitum* food and water and maintained on a 12:12-h light/dark cycle, at 24°C room temperature. All studies were conducted using behaviorally uncharacterized animals from the same breeding colony. Rats of both age-groups were sub-divided randomly into treated (NGF) and untreated (control) groups. Before start of the behavioral test, animals were handled for 10 min/day for 1 week. The behavioral recordings took place between 10:00 and 16:00 h. This study was conducted with approval of the local Ethics Commission (Polish Law on the Protection of Animals) and was carried out in accordance with the Principles of Laboratory Animals Care (NIH Publication No. 86-23).

Surgery

Rats were anesthetized with a mixture of 62.5 mg/kg ketamine and 3.2 mg/kg xylazine dissolved in 0.9% sterile saline. After anesthesia the rats were mounted in a small animal stereotaxic apparatus (David Kopf, Tujunga, CA, USA) with bregma and lambda in the same horizontal plane. In sterile conditions, a burr hole was made 0.92 mm posterior to bregma and 1.6 mm lateral to the midline [63]. A 28-gauge stainless steel cannula (Alzet Brain Infusion Kit, Alza Corporation, Palo Alto, CA, USA) was lowered stereotaxically (4.5 mm dorsoventrally from dura) to the left and right lateral ventricle. The cannula was connected to

subcutaneously implanted osmotic minipump (Alzet 2004, Alza Corporation, Palo Alto, CA, USA) via silastic tubing to secure 1-month continuous delivery of substances. The pumps were filled with 200 μ l per pump either β -NGF (Sigma, NY, USA; NGF groups) or vehicle solution (artificial cerebrospinal fluid containing 100 μ g/ml rat serum albumin; control groups). NGF was diluted in sterile artificial cerebrospinal fluid containing 100 μ g/ml rat serum albumins. A dose of 100 μ g of NGF (a total amount infused) was selected as the 0.5 μ g/ μ l working concentration for intra-ventricular infusion to be used in the experiment. Chloromycetin sodium succinate (chloramphenicol, 1% solution) was applied to the exposed skull and scalp prior to closure to limit local infection; lidocaine was applied locally to the scalp to lessen pain. Antibiotic G Penicillin (biciclin, 300,000 U/ml) and 5 ml of sterile isotonic saline was injected subcutaneously to prevent infection and dehydration during recovery.

Apparatus

The test arena was a black painted square (95 cm \times 95 cm) enclosed by walls (30 cm high) illuminated in the center by 50 W halogen bulb. The OF was surrounded by gray curtains so that the environment was uniform except for a conspicuous striped pattern, 50 cm wide and 35 cm high attached to the wall of the apparatus. A video camera above the field was connected to a video recorder and computer. The objects to be distinguished were made of either glass, plastic, or metal and existed in duplicate. They could not be displaced by the rats. As far as could be ascertained, the objects had no natural significance for rats and had never been associated with reinforcement.

Behavioral procedure

Animals were trained 28 days after surgery immediately after termination of NGF delivery. The cognitive behavior of the young and aged animals was assessed by OLT and ORT. The apparatus and behavioral procedure were similar to those described by Save et al. [64]. Briefly, rats were individually submitted to six successive 5-min sessions, each of which was separated by a 3-min interval during which the rats were returned to their home cage. Sessions 1–4 constituted a familiarization phase. During Session 1, rats were placed into the empty OF for 5 min of habituation. During Sessions 2–4, the objects were placed. Five different junk objects were simultaneously present in the OF. Four of them were located at the corners of an ideal square, while the fifth object being

equidistant from the four others was placed in the middle. In Session 5 (spatial test trial), the configuration was changed by moving two objects to a new location. For the last Session 6 (novelty test trial) one of the familiar objects was replaced by a new differently shaped one. The amount of time spent by each animal for the object exploration was recorded. Exploration was considered to be directing the nose at a distance <2 cm to the object and/or touching it with the nose.

Data collection and processing

Video-based EthoVision System (Noldus, Wageningen, The Netherlands) and additional software (Boguszewski, <http://www.ptbun.org.pl/pmbogusz/behaview/BehaView.exe>) was used to collect and analyze data of OF test. The rats' responses in OLT and ORT were evaluated as discrimination indexes (DIs). *Response to the spatial change* was assessed by comparing the mean time in contact with the displaced (two) objects during Session 5 (OLT test session) and the mean time spent in contact with the remaining (three) non-displaced objects. The following equation was used for displacement discrimination index, DI_D : $DI_D = t_D / (t_D + t_{ND})$, where t_D = mean exploration time of the displaced objects and t_{ND} = mean exploration time of the non-displaced objects. *Response to the new object* was defined as the mean time spent exploring this object during Session 6 (ORT test session) and the mean time of exploration of familiar objects not displaced (two) in Session 5. Data from the objects displaced in Session 5 were not taken into account for the analysis because these objects could acquire a somewhat ambiguous status in terms of familiarity—novelty. The novelty discrimination index, DI_N was calculated as: $DI_N = t_N / (t_F + t_N)$, where t_N = exploration time of the novel object and t_F = mean exploration time of the familiar objects. *Habituation* was assessed by comparing the total time in contact with the objects during Sessions 2, 3, and 4. Additionally, the following *parameters of exploratory behavior* were calculated: (1) distance moved in the peripheral zone of the OF, (2) distance moved in the central zone, (3) number of enters to the center, and (4) the ratio of time in center to the total time of trial.

Histology

Animals were deeply anesthetized with Nembutal and tissues were fixed by intra-aortic perfusion with 0.9% NaCl followed by a mixture of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed and placed for 1 h in the fixative solution and

then immersed for cryoprotection in 10% followed by 20% glycerol + 2% DMSO. Sections were cut coronally through the forebrain at 50 μ m thickness with a freezing stage microtome and Nissl-stained to evaluate minipump cannula placement or were collected free-floating for immunohistochemistry.

Immunohistochemistry

Free-floating consecutive sections of the same animal were processed for choline acetyltransferase and high-affinity NGF receptor (TrkA) immunohistochemistry, using antibodies against ChAT and TrkA (CHEMICON Int.), respectively. Neuronal counts were performed independently for ChAT- and TrkA-immunoreactive neurons. All consecutive sections of each series were analyzed. Counts per section were corrected by Abercrombie's formula to obtain the correct values. Afterwards, the packing density (PD) of cholinergic neurons was calculated as a function of rostro-caudal level and of location within medial septal nucleus and magnocellular basal nucleus by using the determined number of cells and square area of outlined frames in each section analyzed. Details of method are described in our previous publications [10, 90].

Statistical analysis

Morphological data, as well as data of habituation and exploratory behavior were analyzed by two-way analysis of variance (ANOVA) with drug treatment and age as factors. The DIs data were analyzed by non-parametric Kruskal–Wallis ANOVA and multiple comparison *Z*-values. Differences were considered significant when $P < 0.05$. In the recall trials in Sessions 5 and 6, the DI was also compared with chance performance ($DI = 0.5$) by a one-sample Student's *t*-test.

Results

Response to spatial change

Figure 1A shows the difference (reflected by DI_D) between the time spent at Sessions 5 and 4 in the exploration of the displaced objects by young and aged rats treated with either NGF or saline. As can be seen, response to spatial change was improved significantly ($\nu = 3$, $P < 0.01$; non-parametric Kruskal–Wallis ANOVA and multiple comparison *Z*-values) only in aged rats treated with NGF. The response to the

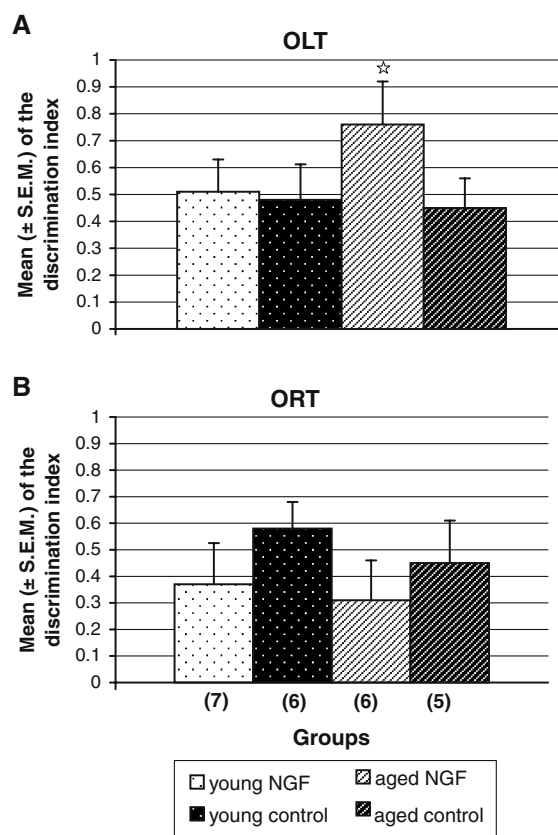


Fig. 1 Effect of nerve growth factor (NGF) administration on behavioral response to the spatial change and object change in young and aged rats. The histograms represent difference between spatial change scores (A) or object change scores (B) defined by discrimination indexes (DIs). DIs were calculated as: $DI_D = t_D/t_{ND} + t_D$, where t_D = exploration time of the displaced object and t_{ND} = exploration time of the non-displaced object, in object-location test, and $DI_N = t_N/t_F + t_N$, where t_N = exploration time of the novel object and t_F = exploration of the familiar object, in object-recognition test. Values are the means (\pm S.E.M.) with the number of animals/group in parentheses indicated under each bar. The asterisk denotes a significant difference of DI in aged NGF-treated rats vs. aged control and young NGF and young control groups; non-parametric Kruskal–Wallis analysis of variance and multiple comparison *Z*-value ($\nu = 3$, $P < 0.01$)

spatial displacement in rats of the remaining experimental groups was rather poor. They tended to fail to discriminate the spatial change regardless of their age (young or aged). The discrimination ratios were lower than the chance level (0.5) in young NGF and young and aged saline rats, however only in aged saline group the difference was significant ($t = 2.94$, $P < 0.01$). In contrast to that of the other groups, in aged NGF-treated rats the mean discrimination ratio ($DI_D = 0.77 \pm 0.14$) was significantly higher from chance performance ($t = 3.70$, $P < 0.01$).

Response to the new object

During Session 6 when a new object replaced a familiar one, all groups of animals reacted by increasing the time spent exploring this object compared with the mean time spent exploring the familiar non-displaced object. Nonetheless, the DI_N for object recognition during Session 6 did not significantly differ between experimental groups. Unexpectedly, the groups treated with NGF, both young and aged, spent less time in contact with the novel object than the saline groups, however this difference was not significant. Kruskal–Wallis ANOVA failed to reveal any significant treatment and age effect ($\nu = 3$, $P < 0.05$). The mean discrimination ratios (DI_N) were not significantly different from chance level (0.5).

Habituation

For all experimental groups total time in contact with the object decreased within Sessions 2, 3, and 4, which indicates that habituation occurred (Fig. 2). This was assessed by a significant effect of sessions [$F(2,40) = 17.23$, $P < 0.00004$] with no significant effect of treatment [$F(1,20) = 0.38$, $P = 0.54$]. Albeit the total time of object exploration in the aged rats was lower than that in the young animals [significant effect of age, $F(1,20) = 16.37$, $P < 0.0006$], they display a normal-like pattern of habituation [no significant age \times sessions interaction, $F(2,40) = 2.66$, $P = 0.08$].

Open-field activity

Open-field exploratory behavior was measured by four different parameters reflecting motor activity and anxiety of each experimental group [65]. The results are presented in Fig. 3. An ANOVA computed on each measure yielded no treatment (NGF vs. saline) effect $F(1,22) = 0.03$, $P = 0.86$ for mean distance moved in the periphery (Fig. 3A); $F(1,22) = 1.45$, $P = 0.25$ for mean distance moved in the center (Fig. 3B); $F(1,22) = 0.38$, $P = 0.54$ for mean number of enters to the center (Fig. 3C); and $F(1,22) = 0.09$, $P = 0.77$ for the mean time in center/total time ratio (Fig. 3D). However, there was a significant effect of age. The mean distance in the center [$F(1,22) = 8.76$, $P < 0.004$], the mean number of entrances to the center [$F(1,22) = 11.73$, $P < 0.0003$], and the mean time in center/total time ratio [$F(1,22) = 13.04$, $P < 0.0001$] were significantly lower in aged rats compared to the young ones. The exception was the mean distance moved in the periphery of the OF which was similar in both age-groups. In comparison with respective con-

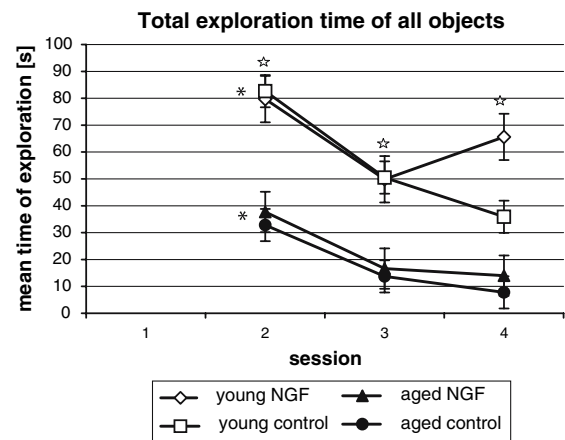


Fig. 2 Effect of nerve growth factor (NGF) administration on habituation course in young and aged rats. Habituation is expressed as change in total exploration time of objects presented in Sessions 2, 3, and 4 (mean \pm S.E.M.). Significant difference among experimental groups and habituation sessions were calculated using three-way analysis of variance (allowed by Newman–Keuls post hoc test). Habituation course was significantly effected by age [$F(1,20) = 16.37$; $P < 0.0006$] and session [$F(2,40) = 17.23$; $P < 0.00004$], however administration of NGF had no significant effect on habituation. The asterisks above the curves denotes a significant (at least $P < 0.001$) difference between young and aged groups, both NGF-treated and controls, at the given session. The asterisks on left denotes a significant (at least $P < 0.02$) difference with respect to the exploration time in Session 2 vs. Sessions 3 and 4 for each experimental group

trols, the groups treated with NGF did not differ in variables reflecting open-field exploratory behavior, indicating no effect of NGF on locomotor activity and anxiety level. However, as can be seen in Fig. 3B–D, the entrances and time spent in the central zone decreased significantly with age, indicating an increase of anxiety in aged rats compared with young controls.

Immunohistochemistry for cholinergic neurons in the basal forebrain

To determine whether cholinergic parameters were changed with age, we evaluated number of neurons expressing the acetylcholine synthesis enzyme ChAT and TrkA receptor of NGF (specific cholinergic neuronal markers) in the BF of young and aged rats. The results of the current experiment are in general agreement with our previous studies indicating that the integrity of BF cholinergic system is deteriorated in aged rats [10, 90]. In the 28-month-old control rats, the PD of ChAT- and TrkA-ir cells was significantly (ANOVA) reduced as compared to the 4-month-old group. Marked changes were observed in both BF structures analyzed (Table 1).

Nerve growth factor inverted the age-induced morphological changes in cholinergic neuron. The

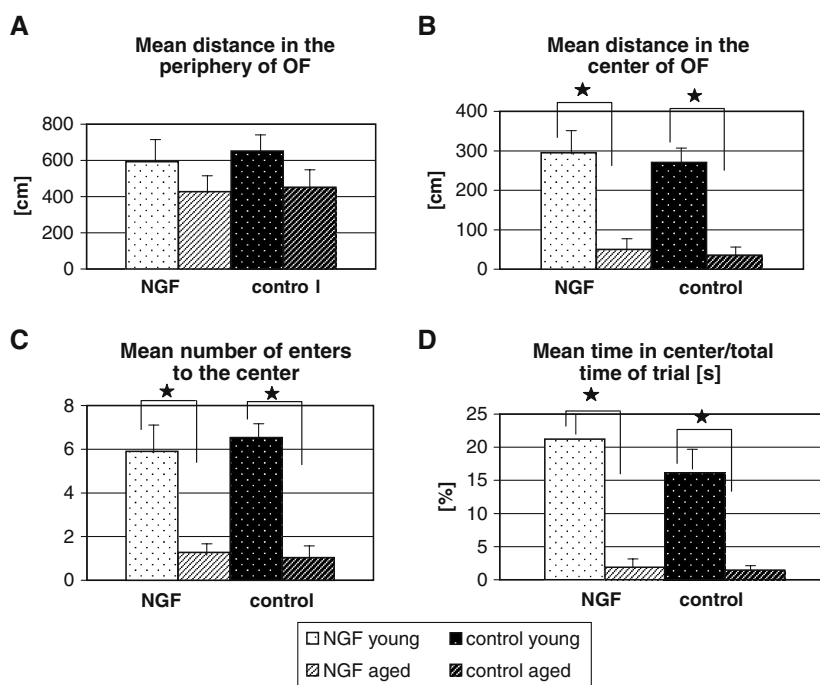


Fig. 3 Factors reflecting spontaneous behavior of nerve growth factor (NGF)-treated and control young and aged rats in open field calculated for all experimental sessions. Mean distance moved in border zone (**A**) did not differ between young and aged rats. Analysis made for three other factors—**B** mean distance moved in central zone, **C** mean number of entries into central zone, and **D** ratio (as percentage) of mean time spent in central

zone to the total time of trials revealed significant difference between young and aged animals. No significant influence of NGF treatment was observed in both age-groups. Difference between experimental groups: effect of age, at least $P < 0.001$; effect of treatment—NS; age \times treatment interaction—NS (two-way analysis of variance for age and treatment performed for each factor presented on the figure)

reduction in the number of ChAT- and TrkA-ir neurons in aged rats was partially or completely reversed by NGF infusion (Table 1).

Discussion

In the present experiment, we used tasks that exploited the rodents spontaneous preference for novel objects, which were first introduced by Ennaceur and Delacour [66]. Paradigm introduced by us [67] has the advantage that, in addition to examining the exploration of a

novel object, it can be used to examine other aspects of recognition, such as object location and context. Thus, both spatial and non-spatial working memory can be tested by using the same paradigm. The major findings of this study are as follows: (1) a bilateral infusion of NGF into lateral ventricles, at the dose used in this experiment, enhanced DI in OLT in aged rats and (2) exogenous NGF had no clear effect on non-spatial memory. Administration of NGF induced a decrease in object recognition, both in young and aged rats, however the difference between the DI of the treated and control animals was not significant. Values of DI

Table 1 Mean (\pm S.E.M.) packing density (as a number of cells/mm²) of neurons immunoreactive for ChAT and TrkA in medial septal nucleus (MS) and magnocellular basal nucleus (MBN) of young and aged rats both controls or nerve growth factor (NGF) treated

Staining	Treatment	MS			MBN		
		Young	Aged	<i>P</i> values	Young	Aged	<i>P</i> values
ChAT-ir neurons	Controls	101.0 \pm 5.1	77.0 \pm 17.8	$P < 0.001^*$	226.1 \pm 24.2	141.4 \pm 17.9	$P < 0.01^*$
	NGF	105.2 \pm 6.9	98.6 \pm 9.1	$P < 0.04^{**}$	255.4 \pm 11.7	256.0 \pm 20.3	$P < 0.002^{**}$
TrkA-ir neurons	Controls	95.6 \pm 4.4	45.7 \pm 9.2	$P < 0.001^*$	209.5 \pm 17.8	60.0 \pm 27.1	$P < 0.0002^*$
	NGF	101.2 \pm 6.3	79.7 \pm 18.7	$P < 0.02^{**}$	235.1 \pm 10.7	163.2 \pm 24.6	$P < 0.0015^{**}$

Two-way (age \times treatment) analysis of variance

* Significant influence of age factor; ** significant influence of treatment factor

for response to spatial change and object change close to chance level indicate that both young and aged animals did not discriminate well either the shift in the spatial location of the object or the introduction of a novel object. The only one exception was the NGF-treated group in OLT for which DI was significantly higher than the chance performance. In the present study, we showed that in aged rats NGF modulated spatial and recognition memory differently. In consequence of NGF infusion aged rats enhanced their responsiveness to spatial change ($DI > 0.5$ in OLT) and avoid the novel object introduced ($DI < 0.5$ in ORT). This aversive tendency was not strong, as the mean DI, however lower did not differ significantly from the chance level. The differential effect of NGF in OLT and ORT could be due to various brain circuits involved. The ORT is controlled mainly by perirhinal–postrhinal cortex and depends much more on mnemonic processes [68–70], whereas response to spatial displacement is controlled by the hippocampus and medial prefrontal cortex [71–74]. It must be noticed, that these later structures receive very strong cholinergic input from the BF nuclei.

These results are inconsistent with that of other studies which have shown that enhancement of cholinergic function improved learning of object recognition in aged [75–77] or transgenic animals [78] impaired in NGF expression. These are many possible causes for these dissimilar results. The main one could be the different behavioral paradigm in which several objects (more than two) presented simultaneously were used. This procedure presented the animals with higher level of difficulty than in other studies. The exact mechanism involved in the different NGF influence on spatial and object recognition in our test remains unclear. We postulate that cognitive processes related to attention are involved. The greater selectivity of attention could be helpful in tests of high difficulty, when the contextual cues are not easy to differentiate [79–81]. Selective attention could interfere positively with performance when subjects are confronted with several simultaneously presented objects and must respond to one while disregarding the others. Thus, we postulated that the reason for the superior performance of NGF-treated aged rats in OLT could be their more efficient attentional processes instead of better learning per se. This hypothesis is consistent with the results of experiments which demonstrated that rats are impaired in the acquisition and retention of high attentionally demanding spatial tasks [82–86]. In addition, studies in rodents [3, 50, 54] and monkeys [42, 54] have demonstrated that selective loss of BF cholinergic nuclei does not disrupt mne-

monic abilities but rather indicate attentional defects in learning and memory tests [36, 87]. Our results may suggest that the very selective difference observed in OLT could be explained by differences in attentional processes which are enhanced due to protective action of NGF on cholinergic system.

The results of earlier observations [65, 88, 89] proved that two non-associative factors, motor activity and anxiety, predominantly influence the exploratory behavior in OF. In our study, we assessed four different variables reflecting either motor activity (mean distance moved in the periphery of OF) or anxiety (mean distance moved in the center of OF, mean number of enters to the center, and mean time spent in the center/total time ratio) in rats tested in OLT and ORT. The comparison of these variables between age-groups showed a significantly higher level of anxiety and similar motor activity in old rats compared with young animals. These data suggest that the behavior of old individuals in the OF was mostly driven by the anxiety component. NGF delivery did not influence significantly any of the variable measured both in young and aged animals. Therefore, the increased level of DI in OLT found in NGF-treated aged rats seems to be the effect of NGF influence on cognitive aspect of learning rather than the action of NGF on non-associative components of learning process.

Compared to the young control brains, a substantial depletion of cholinergic neurons was observed within the BF of all aged brains. Data of ChAT and TrkA immunoreactivity were consistent with that of our previous study [90] and revealed an overall lower ChAT and TrkA staining intensity in cell bodies and in fibers of aged rats. These results suggest a general down regulation of cholinergic activity, as well as diminished TrkA expression and are indicative of a loss of cholinergic markers in the aged BFCN neurons. However, localized NGF infusions proximal to BF cell bodies increase neurochemical parameters of cholinergic neurons in aged rats. Enhanced morphology of cholinergic neurons after administration of NGF correlated with improved performance of aged rats in OLT. This was not observed in young adult animals in which infusion of NGF did not influence either cholinergic markers of BF neurons or learning abilities. It is possible that in aged subjects exogenous NGF could overcome the deficient retrograde transport of target-derived neurotrophin and protect the animals from developing age-related deficits in cholinergic projection and memory function.

In conclusion, the data suggest that, in aged rats, NGF positively effects cognitive processes related to

spatial memory and attention, but is ineffective on memory processes involved in the formation of associations established in recognition memory tasks, which require higher neural integration. These findings, together with the lack of effect of NGF on motor behavior and anxiety, argues in favor of a very specific action exerted by NGF on mnemonic functions, which depends on the nature of the task and the cognitive processes involved in formation of memory. The treatment with exogenous NGF which increased the cholinergic markers in BF recorded in morphometric analysis [10, 90, 91] did not result in a general improvement of learning in aged subjects. Together, this body of evidences supports the potential utility of NGF in the treatment of memory disorders, however its cognitive-enhancing properties seem to be limited.

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