Chronic nicotine improves working and reference memory performance and reduces hippocampal NGF in aged female rats

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Abstract

The cholinergic system is involved in cognition and several forms of dementia, including Alzheimer’s disease, and nicotine administration has been shown to improve cognitive performance in both humans and rodents. While experiments with humans have shown that nicotine improves the ability to handle an increasing working memory load, little work has been done in animal models evaluating nicotine effects on performance as working memory load increases. In this report, we demonstrate that in aged rats nicotine improved the ability to handle an increasing working memory load as well as enhanced performance on the reference memory component of the water radial arm maze task. The dose required to exert these effects (0.3 mg/kg/day) was much lower than doses shown to be effective in young rats and appears to be a lower maintenance dose than is seen in light to moderate smokers. In addition, our study reports a nicotine-induced reduction in nerve growth factor (NGF) protein levels in the hippocampus of the aged rat. The effects of nicotine on hippocampal NGF levels are discussed as a potential mechanism of nicotine-induced improvements in working and reference memory.

Keywords: Nicotine; Working memory load; Aging; Neurotrophin; Water radial arm maze

1. Introduction

Many age-related neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) involve cognitive dysfunction. Both the nicotinic and muscarinic components of the cholinergic system have been implicated in AD and age-related cognitive impairment [38]. High affinity nicotine binding is decreased in several conditions which manifest cognitive impairment and/or dementia, including AD, PD, Lewy body disease, and Down’s syndrome, particularly in brain areas related to the pathophysiology of these diseases [30,31]. Interestingly, nicotine has positive effects on cognition in AD patients (a population that exhibits both working and reference memory deficits), in human volunteers with experimentally induced cognitive impairment (e.g., by administration of alcohol, scopolamine, or sleep deprivation), and in healthy adults [27,33,39,40]. These and other studies suggest that nicotinic acetylcholine receptors (nAChRs) are an important target in treating cognitive impairment.

In animal studies, nicotine has been shown to improve reference memory performance of aged rats in the Morris water maze (MWM) and the land radial arm maze (RAM) [5,34,37]. On the other hand, while chronic nicotine did improve working memory in young rats in the land RAM, it did not improve working memory performance in aged rats [5,25]. The null effects in the latter study could be explained by the fact that the data were collapsed across all choices within a session, potentially masking effects of nicotine at different working memory loads as trials progressed. Furthermore, although the dose examined by Levin and Torry (5 mg/kg/day via osmotic pump) was effective in young rats [25], it is possible that different doses are required to improve cognitive performance in aged rats. In fact, lower doses may be more desirable given that Okamoto et al. showed decreased metabolism of nicotine in aged rats [28].
In Olton and Samuelson’s landmark paper using the land RAM, they noted a decrease in performance as trials progressed and working memory load increased within each session [29]. However, since their report, little work in young or aged rats has been done that specifically addressed working memory load. Recently, Rimone et al. compared the ability of young and aged rats to handle an increasing working memory load using the water RAM and found that aged rats are less able to handle numerous items of information [9–12]. Another group reported nAChR antagonist-induced working memory deficits at a high working memory load; however, this study did not compare performance across trials as working memory load increased [1]. Since human studies have shown that nicotine has specific effects on ability to handle an increasing working memory load [14,18,24], this is an important cognitive component to examine when evaluating drugs targeting the nicotinic cholinergic system.

In addition to its cognitive effects, there is direct evidence that nicotine is neuroprotective [35,36]. This neuroprotection could be mediated through nicotinic regulation of expression of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and their receptors [15,16,22,23]. It has been reliably demonstrated that NGF and BDNF play important roles in cognitive ability and in maintenance of cholinergic neur- ons of the basal forebrain [6,9,13,26]. Thus, the neurotransph system is an important potential target of nicotine in the brain.

In the present study, we investigate whether chronic nicotine improves the cognitive performance of aged female rats. Low doses of nicotine (0.1 and 0.3 mg/kg/day) were chosen for our study based on data suggesting that nicotine metabolism is decreased in aged rats [28]. Chronic administration was chosen to more closely mimic clinically relevant delivery systems such as the nicotine transdermal patch. The water RAM allows us to assess working and reference memory performance simultaneously as well as to examine the effects of nicotine as working memory load increases. Given the links between nicotine, memory, and the neurotransph system, we also examine effects of nicotine on NGF and BDNF in specific brain regions linked to cognition and evaluate whether these changes correlate with maze performance.

2. Materials and methods

2.1. Subjects

Subjects were 31 24-month-old (aged) Fisher-344 female rats born and reared at the aging colony of NIA at Harlan Laboratories (Indianapolis, IN). The subjects were divided into three treatment groups matched by body weight. One subject was excluded from all analyses due to extremely high BDNF levels in the frontal cortex (>3 standard deviations (S.D.) from the mean). Treatment groups and number of subjects were as follows: aged saline vehicle (aged control; n = 11), aged 0.1 mg/kg/day nicotine (aged 0.1 mc; n = 10), and aged 0.3 mg/kg/day nicotine (aged 0.3 mc; n = 9).

2.2. Chronic administration of nicotine using osmotic mini-pumps

Nicotine ditartrate (Sigma) in 0.9% sterile saline or vehicle alone was administered to subjects subcutaneously using Alzet® osmotic mini-pumps (Durect, Cupertino, CA). The concentration of nicotine in the pumps was calculated to deliver 0.1 or 0.3 mg/kg/day nicotine base. Pumps were prepared the day before surgery and allowed to equilibrate at 37 °C overnight. Prior to pump insertion surgery the subjects were anesthetized with a cocktail of 70 mg/kg ketamine and 6 mg/kg xylazine delivered by i.p. injection. Recovery from anesthesia occurred under a heat lamp with monitoring of body temperature. The pumps were implanted at least 1 week after receipt of the subjects and remained in the subjects throughout behavioral testing and until sacrifice (approximately 4 weeks).

2.3. Body weight

Body weight was monitored before and after implant surgery and weekly thereafter until the end of testing. These data were analyzed using a 1-Between (Treatment) 1-Within (Time) repeated measures analysis of variance (ANOVA). In addition, a 1-Within (Time) repeated measures ANOVA for each individual group was performed to determine whether each group lost weight during the study.

2.4. Plasma cotinine analysis

Cotinine plasma concentrations were assessed in order to evaluate whether nicotine was received and metabolized by the subjects via osmotic pumps. Cotinine is a metabolite of nicotine used to estimate nicotine exposure in animal subjects and in humans, and it is not present in untreated subjects. Cotinine plasma concentrations were determined using the cotinine assay kit from Ora- sure (Burlington, PA). Blood was collected via heart puncture in heparinized syringes at the time of brain dissection. Samples were centrifuged for 15 min at 1800 x g at 4 °C to separate the plasma, and plasma was stored at −20 °C until evaluation. Commercial plates coated with a primary capture antibody were supplied by the cotinine kit. Samples and horseradish peroxidase-conjugated cotinine were added to the coated plate and incubated in the dark at room temperature. After washing to remove excess conjugate, the enzyme substrate was applied and absorbance was measured at 450 and 630 nm in a spectrophotometer. A standard curve was created from cotinine standards provided with the kit. Two-tailed t-tests were performed for two-group comparisons of interest.

2.5. Water-escape radial-arm maze

Working and reference memory were assessed simultaneously using the 8-arm water radial maze, which has been used routinely in our and other laboratories [7,10,12,17]. The maze was constructed of galvanized steel, painted black, placed in a room with salient extra-maze cues, and filled with room tem- perature water. Escape platforms were hidden 1 cm below the water level in the ends of four of the eight arms. The testing procedure has been described in detail previously [8,9]. Briefly, each subject had platform locations that remained fixed throughout the experiment. For each trial a subject was released from the start arm and had 3 min to locate a platform. If the subject had not located the platform in 3 min, the experimenter guided it to the closest platform. Once a platform was found the trial was over, the subject remained on the platform for 15 s, and then was returned to its heated cage for a 30 s intertrial interval (ITI). During the ITI, the just-chosen platform was removed from the maze. A daily session consisted of this sequence of events repeated until all four platforms were located, read- ing in four trials per session. Each subject received one session per day for 15 days, with 2-day breaks given after days 5 and 10. An arm entry was counted when a subject’s snout reached a mark delineated on the outside of the maze (11 cm into the arm). This study was conducted in two waves with all treatment groups equally represented in each wave. This is the typical procedure for our laboratory to make behavioral testing feasible [9,12].

2.6. Error quantification

As done previously with the water RAM, working memory correct (WMC), reference memory (RM), and working memory incorrect (WMI) errors were...
whether significant weight loss occurred due to nicotine treatment. This indicates that nicotine treatment did not influence weight loss.

3.1. Weight analysis

Weight was monitored throughout the study to determine whether significant weight loss occurred due to nicotine treatment (Fig. 1). There was a significant Time effect for Weight when all groups were collapsed [F(4, 116) = 105.570, p < 0.001], which held for each individual treatment group (aged control [F(4, 40) = 51.276, p < 0.001]; aged 0.1 nic [F(4, 36) = 37.495, p < 0.001]; aged 0.3 nic [F(4, 32) = 20.392, p < 0.0001]). In other experiments within our laboratory we have noted weight loss in aged rats due to behavioral testing (unpublished observations). Such effects may be attributable to exercise or a multitude of independent or interactive variables which remain to be tested in future evaluations. In this study, there were no main effects of treatment (all p’s > 0.80) or treatment × time interactions (all p’s > 0.10) between groups for body weight, indicating that nicotine treatment did not influence weight loss during the study. This indicates that the doses of nicotine administered did not cause substantial loss of appetite or wasting, an important consideration in using nicotine to treat cognitive impairment in the aged population.

3.2. Cotinine plasma levels

As expected, cotinine was not found above detection limits in the aged control subjects, whereas levels were elevated in the two nicotine treatment groups. Cotinine levels differed between the treatment groups (aged ctrl versus aged 0.1 nic [t(19) = 7.737, p < 0.001]; aged ctrl versus aged 0.3 nic [t(18) = 8.919, p < 0.0001]; aged 0.1 nic versus aged 0.3 nic [t(17) = 6.278, p < 0.0001]). These plasma cotinine levels confirm that nicotine was delivered to the subjects successfully. The cotinine levels observed for the nicotine doses used in this study (0.1 mg/kg/day nic: 15.2 ± 6.4 ng/ml; 0.3 mg/kg/day nic: 64.7 ± 24.1 ng/ml), were below those seen in light to moderate smokers (84.5 ng/ml plasma cotinine, 1–4 cigarettes/day; 167.2 ng/ml plasma cotinine, 5–9 cigarettes/day) [20].

3.3. Behavior analysis

3.3.1. Learning

Fig. 2 shows the number of working memory correct, reference memory, and working memory incorrect errors made during the initial (days 2–6) versus latter (days 7–15) phases of testing, averaged across days. A significant decrease in errors from one phase to the next is indicative of learning. The aged control group showed significant learning for WMc [F(1, 10) = 8.499, p < 0.05] and showed marginal improvement for WMi [F(1, 10) = 4.276, p = 0.07]. The aged 0.3 nic group showed learning on all three measures (WMC [F(1, 8) = 26.905, p < 0.001]; RM [F(1, 8) = 155.001, p < 0.0001]; WMI [F(1, 8) = 23.569, p < 0.005]), while the aged 0.1 nic group only improved RM and WMI performance across phases (RM [F(1, 9) = 12.959, p < 0.01]; WMI [F(1, 9) = 11.929, p < 0.01]).
Fig. 2. Working memory correct (WMC), reference memory (RM), and working memory incorrect (WMI) errors averaged over days ± S.E. are blocked by initial (I – days 2–6) and latter (L – days 7–15) phases of testing for each treatment group. Within group comparisons between phases indicate whether learning occurred. For WMC, only the aged 0.3 nic group showed significant learning; for RM, all three treatment groups showed significant learning; for WMI, both nic treatment groups showed learning. Significant between group comparisons of the treatment effect for the latter phase of testing are also indicated: the aged 0.3 nic group performed better than aged controls on all three measures, and the aged 0.3 nic group out-performed the aged 0.1 nic group on RM. These results indicate that the aged controls had difficulty learning the working memory components of the task. Further, nicotine, particularly at the 0.3 mg/kg/day dose, improved ability to learn both working and reference memory components of the task in aged rats.

3.3.2. Initial phase of testing (days 2–6)
Analysis of days 2–6 showed a marginal working memory incorrect treatment × trial interaction between the aged 0.3 nic and aged control groups \( F(3, 54) = 2.658, p < 0.057 \) with aged controls making more errors than aged 0.3 nic subjects as trials progressed (data not shown). There were no significant main effects of treatment for any two-group comparisons for days 2–6.

3.3.3. Latter phase of testing (days 7–15)
As shown in Fig. 2, the aged 0.3 nic group consistently made fewer errors than the aged control group, showing better performance for all three measures during the latter phase of testing (WMC \( F(1, 18) = 6.935, p < 0.05 \), RM \( F(1, 18) = 13.317, p < 0.005 \), WMI \( F(1, 18) = 8.159, p < 0.05 \)). In addition, for RM, the aged 0.3 nic group performed better than the aged 0.1 nic group \( F(1, 17) = 7.568, p < 0.05 \). Further, since the aged 0.1 nic group was not significantly different than the aged control group, it appears that the 0.1 nic treatment was not sufficient to impact RM. Aged 0.1 nic and aged control groups did not differ for the WMC or WMI main effect of treatment (e.g., collapsed across all trials).

3.3.4. Working memory load
Fig. 3 shows the number of working memory correct and incorrect errors made by each group for each trial collapsed across days 7–15. For WMC, there was a treatment × trial interaction between the aged 0.3 nic and aged control groups \( F(2, 34) = 5.185, p < 0.05 \) and between the aged 0.3 nic and the aged 0.1 nic groups \( F(2, 34) = 3.991, p < 0.05 \), with the aged 0.3 nic group making fewer errors than the other groups as trials progressed. For WMI, treatment × trial analyses showed differences between the aged control group and both aged 0.3 nic \( F(3, 54) = 7.234, p < 0.0005 \) and aged 0.1 nic \( F(3, 57) = 2.655, p < 0.05 \), with the nicotine groups showing enhanced performance. Collectively, these findings suggest that nicotine treated subjects were better able to handle an increasing working memory load.

3.4. Growth factors
NGF and BDNF protein levels were assayed in samples dissected from the basal forebrain, frontal cortex, hippocampus, and entorhinal cortex. As seen in Fig. 4, hippocampal NGF levels were lower in the aged 0.3 nic group compared to the aged control group \( t(18) = 2.240, p < 0.05 \). No significant effects of nicotine treatment were seen for NGF in the basal forebrain, frontal cortex, or entorhinal cortex, or for BDNF in any brain region. No intraclass (within group) or interclass (across groups)
Fig. 4. Nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) protein levels (mean ± S.E.) were assayed in hippocampus, basal forebrain, entorhinal cortex, and frontal cortex. (A) Aged 0.3 nic subjects have reduced hippocampal NGF levels relative to aged controls. NGF levels did not differ between treatment groups in basal forebrain, entorhinal cortex, or frontal cortex. (B) There were no significant treatment effects on BDNF levels in any of the four regions examined.

4. Discussion

Although there has been extensive study of nicotine effects on memory in both humans and in animal models [32], little work has specifically evaluated effects of nicotine on performance as working memory load increases. In this report we demonstrate for the first time that nicotine improves the ability of aged female rats to handle an increasing working memory load while performing a spatial memory task. This finding is in accordance with human studies showing that smokers, non-smokers, and schizophrenic patients given nicotine exhibit marked improvements in memory performance at higher working memory loads [14,18,24]. In addition, we found that nicotine improves the performance of aged female rats on the reference memory component of the water RAM. These nicotine-induced memory improvements correspond with our novel finding of a nicotine-induced reduction in hippocampal NGF protein levels in the aged female rat. Interestingly, all of these effects were dependent on the dose of nicotine administered. Only the highest dose examined (0.3 nic) enhanced the ability to handle an increasing working memory load for both orthogonal working memory measures, improved reference memory, and altered hippocampal NGF levels, while effects of the lower dose (0.1 nic) were limited to improving only one working memory measure.

All groups in this study exhibited learning for reference memory, yet only the higher dose group (aged 0.3 nic) performed better than the aged control group during the latter phase of testing for reference memory. In fact, the higher dose group also performed significantly better than the lower dose group (aged 0.1 nic) on this measure. The higher dose of nicotine also consistently improved performance across both working memory measures (WMC and WMI). This was evident both as an improvement in learning across phases and as significantly better performance compared to aged controls during the latter phase of testing.

To further investigate the effect of nicotine on working memory we analyzed performance as trials progressed and working memory load increased. This also allowed an indirect evaluation of motivational changes with nicotine treatment. If motivation were a factor, we would expect to see differences between groups at every trial and no trial × treatment interaction. However, the positive effects on working memory were due primarily to differences seen on trial 4 (when working memory load was highest), resulting in significant trial × treatment interactions. On the other hand, the three groups performed similarly on the earliest trials, when the motivator was the same as for trial 4 but working memory load was lower. The higher dose group consistently demonstrated an enhanced ability to handle an increasing working memory load compared to aged controls for both WMC and WMI. In contrast, the lower dose group performed better than aged controls as working memory load increased for WMI only.

correlations between NGF or BDNF levels and WMC, WMI, or RM performance were found (data not shown).
A possible mechanism for nicotine’s effects on working and reference memory may be related to our novel finding of a nicotine-induced decrease of NGF protein in the hippocampus. These data agree with a previous study in which we found that aged rats whose working memory performance was improved by testosterone treatment had decreased hippocampal NGF levels compared to untreated aged rats [12]. In addition, we have also shown that treatment of reference memory impaired aged rats with exogenous NGF lowered hippocampal NGF levels to those found in unimpaired aged rats [2]. Our findings disagree with a recent study reporting no nicotine-induced changes in hippocampal NGF levels in young male rats; however, this apparent discrepancy could be due to differences in age and/or gender of the subjects, as well as the dose given [16]. Based on the current theory of impaired retrograde transport of NGF from target tissues to cholinergic cell bodies in the basal forebrain, it is plausible that NGF accumulates in the aged hippocampus because retrograde transport is impaired [2,26]. Our finding of nicotine effects on hippocampal NGF may indicate that nicotine administration helps to restore retrograde transport, and this may be related to the nicotine-induced enhancements in working and reference memory performance seen in the current study. Since it is not possible to differentiate intracellular stores from synaptic levels of NGF in our assay, there may be different explanations for the possible role of nicotine in altering hippocampal NGF levels. For instance, if endogenous ACh release is compromised during aging, nicotine may be acting through presynaptic α7 nAChR to increase ACh release at the synapse, which in turn would lead to increased release of NGF from hippocampal target cells, making it more available for retrograde transport. On the other hand, nicotine administration may be upregulating trkA receptor expression on cholinergic neurons projecting to the hippocampus, resulting in increased uptake of NGF at hippocampal synapses and repair of impaired NGF retrograde transport to the basal forebrain. Interestingly, a recent report demonstrated that nicotine treatment increased trkA expression in the hippocampus of young adult male rats [16]. Future studies are needed to further elucidate the effects of nicotine on cholinergic neurons in the basal forebrain and hippocampus of the aged rat.

Our findings of nicotine-induced improvements on working memory differ from the two other studies reporting no effects of nicotine on working memory in aged rats [5,25]. Several factors may be involved in this apparent contradiction. First, while our study evaluated performance as working memory load increased, the other studies collapsed nicotine effects across all choices (trials), possibly masking nicotine-induced improvements at the highest memory load within a session. Indeed, we report working memory effects of nicotine as trials progressed, and there were no differences between groups on early trials. In fact, in our study the lower dose’s enhancement of WMI performance as trials progressed was not evident when data was collapsed across trials. Second, there may be dosing or administration issues involved. Based on work suggesting that nicotine is metabolized less extensively in aged rats than in young rats [28], we administered lower doses (0.1 and 0.3 mg/kg/day via osmotic pumps) to the aged rats in this study than are typically used. For instance, Levin and Torry [25] administered 5 mg/kg/day nicotine via osmotic pumps and Arendash et al. [5] gave daily injections of 0.2 mg/kg nicotine shortly before testing each day. Further, our own study shows different effects on working and reference memory measures depending on nicotine dose. Finally, the land RAM typically requires food deprivation, a potential confound in studies involving nicotine and/or aged rats. Aged female F344 rats (24–25 months) were found to be less motivated by food than their young counterparts (7–11 months) at the same food deprivation levels [4]. In addition, nicotine is known to suppress appetite in humans and rats [21]. The water RAM uses water-escape as a motivator, thus avoiding the potential confound of food deprivation and reward.

In summary, this is the first study to report improvement of working memory in aged rats treated with nicotine and the first evaluation of the interaction between nicotine and working memory load in rats. We also report that while the higher dose of nicotine improved both reference memory and the ability to remember more items of working memory information, the lower dose examined improved working memory without affecting reference memory. Both doses maintain lower plasma cotinine levels than are seen in light to moderate smokers, and the chronic dosing mimics the transdermal patch administration method. In addition, we showed a decrease of NGF in the hippocampus of aged rats with chronic nicotine treatment. Interestingly, this nicotine-induced decrease in hippocampal NGF was only evident at the nic dose shown to result in the most profound cognitive performance enhancements (0.3 mg/kg/day nic). Because nicotine has also been shown to improve working memory performance in humans as working memory load increases, this study presents a valuable model for studying the mechanisms of this interaction. Exploring the relationship between nicotine effects on working/reference memory and nAChR expression, as well as other markers of cholinergic phenotype (e.g., ChAT, AChE, and trk receptor expression), will help to elucidate mechanisms in the nicotinic cholinergic system which could be targeted for novel treatments of neurodegeneration.

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References


