

Effects of nicotine administration on amyloid precursor protein metabolism, neural cell genesis and acquisition of spatial memory

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Abstract

Nicotine is reported to improve learning and memory in experimental animals. Improved learning and memory has also been related to increased neural cell genesis in the dentate gyrus of the brain hippocampal formation. Stimulation of acetylcholine receptors has also been found to enhance the expression and secretion of amyloid precursor protein (APP) in various cell lines. Aberrant metabolism of APP generates amyloid-beta (Abeta) peptide which is the major pathological lesion found in the brains of Alzheimer's disease (AD) patients. This paper will focus on the results obtained in our laboratory on the effects of acute and chronic nicotine administration on the metabolism of APP, its role in spatial learning and neural cell genesis in the rat brain.

Keywords

nicotine, amyloid precursor protein, neurogenesis, spatial learning, hippocampus..

Introduction

There is evidence from both clinical and preclinical studies that nicotine, the principal psychoactive component of tobacco smoke^{1,2}, elicits improvements in cognitive function.³ These effects are thought to be related to stimulation of neurotransmitter systems within areas of the brain that are important for cognitive processing.⁴ As a result, nicotine and nicotinic drugs have been explored for their efficacy as putative treatments for the impaired cognitive function experienced by patients with conditions such as AD.⁵

The subgranular zone of the dentate gyrus is one of the few areas of the brain in which neural cell genesis (neurogenesis) continues to occur into adulthood⁶ with formation of new cells arising from the proliferation of progenitor cells. Increased neurogenesis can be produced by a variety of treatments including an enriched environment⁷, physical activity⁸ and antidepressant drugs.⁹ It has also been specifically implicated in learning tasks that involve the hippocampus. Training rats on a hippocampal-dependent associative task leads to an increase in survival of new-born cells¹⁰ whereas preventing cell proliferation by administering a cytotoxic agent impairs performance.¹¹

The amyloid precursor protein is a glycoprotein that can exist in both membrane-bound (particulate) and secreted forms.¹² The particulate form of the protein (APPp) plays a key role in the modulation of neuronal plasticity, synapse stabilisation and memory consolidation.¹³ The secreted (soluble) form of the protein, APPs, is normally generated by cleavage of the protein by the alpha-secretase protease enzyme at a site proximal to the cell membrane. This form of the protein exhibits both neurotrophic and neuroprotective properties.¹⁴ However, aberrant proteolytic cleavage of APP by beta- and gamma- secretases produce amyloid-beta (Abeta) peptide which is the major

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component of the senile plaques found in the brains of AD patients.¹⁵

Various studies suggest that nicotine enhances neuroprotective mechanisms including protecting against nerve growth factor deprivation¹⁶, glutamate-induced neurotoxicity¹⁷ and Abeta-mediated cytotoxicity.¹⁸ Nicotine exposure increases the number of nAChRs both *in vitro*¹⁹ and *in vivo*²⁰, possibly acting to attenuate the cognitive deficits found in AD associated with a decrease in the levels of acetylcholine due to the degeneration of cholinergic neurons.

Previous studies have shown that a number of neurotransmitter systems, when activated, are able to regulate APP and Abeta metabolism. Of these, the cholinergic system was the mostly studied as loss of cholinergic receptors is an important pathological hallmarks in AD.²¹ The role of nAChR stimulation in the processing of APP has been reported in various cell lines.^{22,23,24} *In vivo*, chronic nicotine administration has been demonstrated to increase APP mRNA levels in the amygdale and hippocampus.²⁵ The present studies used complimentary *in vivo* and *in vitro* experimental approaches to investigate the effect of chronic and acute nicotine treatment on APP expression in different areas of the rat brain and its role in the acquisition of spatial memory and neural cell genesis.

Materials and methods

1. Effects of nicotine on APP metabolism

For the *in vivo* studies, twenty-four young (6-8 weeks, 250-300 g) and eighteen old (18 months, 560-640 g) male Sprague-Dawley rats (Harlan Industries, UK) were used. The animals from each age group were divided into three groups, consisting of controls ((*n* = 8 (young), *n* = 6 (old)), low-dose group that received 0.25 mg/kg nicotine per day ((LDN); *n* = 8 (young), *n* = 6 (old)) and high-dose group that received 4.00 mg/kg nicotine per day ((HDN); *n* = 8 (young), *n* = 6 (old)). The rats were housed, two per cage, at a temperature and humidity controlled environment on a 12-h light/dark cycle and with *ad lib* access to food and water.

Two-week duration osmotic minipumps (Alzet®, ALZA Corporation, Palo Alto, CA, USA) were filled with nicotine (dissolved in 0.9% saline solution) or saline as instructed by the manufacturer. They were implanted subcutaneously in the flank under halothane anaesthesia (5% induction, 3% maintenance) through a small incision on the back at the level of the shoulders. The minipumps were left for 13 days, after which the rats were killed by cervical dislocation followed by decapitation. The brain was removed and the cerebral cortex, striatum and

hippocampus were dissected on ice and stored at -80°C prior to analysis.

Thawed tissue was homogenised for 25-30 s in 10% lysis buffer in addition to a cocktail of protease inhibitors. The insoluble homogenate was removed by centrifugation at 1,500 r.p.m. for 5 min at 4°C. The supernatant was further centrifuged at 14,500 r.p.m. for 30 min. The resulting aqueous phase, composed primarily of the cytosolic fraction, and the pellet, containing the nuclear, mitochondrial and plasma membrane (particulate) fractions were both stored at -20°C until used for APP and APPs protein analysis.

For the *in vitro* studies, hippocampal tissue slices from young (6-8 weeks) and old (19-21 month) Sprague-Dawley rats were used. The rats were killed by cervical dislocation and 400 µm thick hippocampal tissue slices were prepared using a McIlwain tissue chopper. These were added to oxygenated Krebs's buffer solution in addition to a cocktail of protease inhibitors and incubated at 37°C. The contents were centrifuged at 2,500 r.p.m. for 10 min at 4°C. The supernatant was collected for further analysis and stored at -20°C until use.

The protein components of the supernatant were precipitated by the addition of 50% trichloroacetic acid and the tubes incubated at 4°C for 45 min. The contents were then centrifuged at 14,000 r.p.m for 20 min at 4°C to generate a protein pellet.

The protein concentrations of the samples were determined spectrophotometrically using the method of Bradford.²⁶ Samples containing 25 µg of protein were loaded onto 7.5% SDS-PAGE followed by transfer to polyvinylidene difluoride membranes. After blocking for one hour, the membranes were incubated overnight with primary antibody 22C11 (recognizing APP; Chemicon International, UK; 1:1500) at 4°C. After washing, the membranes were incubated with secondary antibody (goat anti-mouse IgG-horseradish peroxidase, Scottish Antibody Production Unit; 1:1500) for 3-h at room temperature and protein bands visualised by enhanced chemiluminescence.

2. Effects of nicotine on spatial learning and neural cell genesis

Male Sprague-Dawley rats (Harlan Industries, UK) weighing 270-340 g at the start of the experiment were used. Rats were housed, three per cage, in a temperature (21°C) and humidity (50±10%) controlled environment on a 12 hour light/dark cycle. Rats were divided into 3 groups: a control group that received

saline (Control, C), low-dose group that received 0.25 mg/kg nicotine per day (LDN) and a high-dose group that received 4 mg/kg nicotine per day (HDN). Nicotine or saline was administered subcutaneously via osmotic minipump and surgically implanted as described above. Following a two-day recovery period, half of the rats were assigned to receive spatial training (ST) and the other half remained in their home cage (NST; no spatial training).

For the spatial learning task, rats were trained in an open field Morris water-maze.²⁷ Rats were trained for 4 days to find an escape platform hidden below the surface of the water. For half of the rats the platform was located in the northeast quadrant and for the other half in the southwest quadrant. Every day rats in the ST groups were given 2 blocks of training composed of 4 trials each. A maximum search time of 120 sec was allowed for each trial. On the day following the last day of training, a probe trial was conducted to assess retention of the platform location by removing the platform and allowing each rat to swim for 60 sec. The rats were tracked using an overhead video camera to allow offline analysis. Performance during task acquisition was assessed from escape latencies (time taken to find the platform). For the probe trial, the computer calculated the percentage time spent in four equal quadrants of the water-maze and the number of times rats swam over the exact position of the platform (annulus crossings).

Measurement of neural cell genesis was carried out using BrdU (Sigma, UK) which integrates into DNA during the S phase of DNA synthesis²⁸. BrdU was dissolved in 0.9% saline and administered (50 mg/kg, i.p.) immediately following the last trial of the first block of training on days 5, 6 and 7. Rats that did not undergo spatial training (NST) were injected at identical time points. On day 10 of the experiment, the rats were deeply anaesthetised with an overdose of i.p. pentobarbital sodium and perfused transcardially with 40 ml of saline followed by 140 ml of ice-cold 4% paraformaldehyde. Coronal sections (20 µm) were cut throughout the hippocampus using a cryostat. Every eighth section was thaw mounted on slides.

The neuronal nuclear protein marker (NeuN) was used to help visualise the neurones within the hippocampus and identify the outer border of the granule cell layer. Slides were coded before counting to ensure objectivity. BrdU-labelled cells were visualised using a fluorescent microscope (Zeiss Axioskop II). All BrdU-labelled cells within the granule cell layer and hilus of the DG were counted and the number of BrdU-positive cells for each subject was expressed as a mean per section.²⁹

Data analysis

For APP measurement, the optical density (OD) of the immunoreactive bands of particulate and secreted forms of APP were analysed quantitatively using the public-domain Scion Image software (Scion Corporation, USA). For the *in vivo* studies, the mean OD was determined for the control groups and the individual values expressed as percentages. To determine any brain regional effects in the *in vivo* studies, a one-way ANOVA with repeated measures was used. Post-hoc analysis of significant effects was performed using Dunnett's test. Time-course release profiles of APPs from control and nicotine-treated hippocampal tissue slices were analysed by repeated measures ANOVA. Profiles of APPs release from hippocampal tissue slice in the presence of nicotine, mecamylamine and EGTA were analysed using two-way ANOVA.

ANOVA with repeated measures was also used to determine group differences in behavioural performance, both during the acquisition of the spatial learning task and probe trial. Post-hoc analysis was carried out using further ANOVA or Dunnett's multiple comparisons test. Group differences in BrdU-labelled cells in the dentate gyrus were analysed using two-way ANOVA and post-hoc using Dunnett's tests.

The level of statistical significance was taken as $p < 0.05$ and the analysis carried out using the Statistical Package for Social Scientists (SPSS, version 11.5).

Results

1. *In vivo* effects of nicotine on APP metabolism

Chronic administration of nicotine increased APPp immunoreactivity in the hippocampus of young ($F[2, 20] = 10.18, p < 0.01$) but not the older rats (Figure 1). The nicotine infusions had no significant effects on APPp immunoreactivity in the striatum or cerebral cortex. Post-hoc tests revealed that the infusion of both of the doses of nicotine tested resulted in increased hippocampal APPp immunoreactivity ($p < 0.01$ and $p < 0.05$ respectively for the rats infused with 0.25 mg/kg/day and 4.00 mg/kg/day). APPs immunoreactivity was not changed significantly by infusions of nicotine in any of the brain regions examined in both young and old rats (data not shown).

2. *In vitro* effects of nicotine on APP metabolism

In order to determine the time course effects of nicotine on APPs release, hippocampal slice preparations from young rats were incubated for periods of up to 2 h in the presence of 0 (control), 1.0 and 10 µM nicotine. Repeated measures ANOVA

showed that nicotine significantly increased APPs levels from hippocampal slices over the two hour incubation time ($F[2, 12] = 4.76, p < 0.05$) (Figure 2). Post-hoc comparisons revealed a significant difference between the control and the hippocampal slice group incubated with the lowest ($p < 0.05$) but not the highest nicotine concentration suggesting the effect of the drug was drug concentration-related but not drug concentration-dependent (Figure 2). Further time-point analysis by one-way ANOVA showed that the greatest APPs release from hippocampal slices was obtained at 1 h incubation period ($p < 0.01$). No significant changes in APPp were observed during the 2 h incubation period (data not shown).

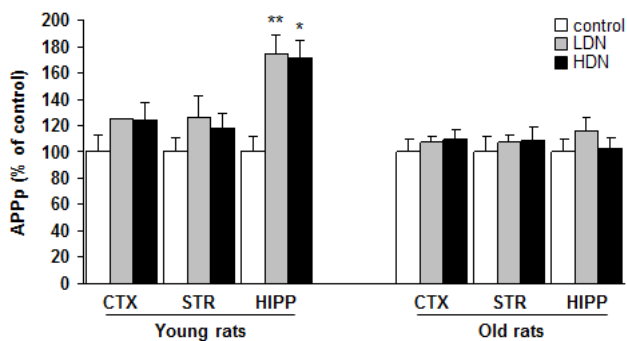


Figure 1: The effects of *in vivo* nicotine infusion on APPp immunoreactivity in the brain of young and old rats. APPp immunoreactivity in the cerebral cortex (CTX), striatum (STR) and hippocampus (HIPP) are presented as percentages (+SEM) of the mean control value ($n = 6$ rats per group) for animals chronically infused with saline (control) or nicotine at doses of 0.25 mg/kg/day (LDN) or 4.00 mg/kg/day (HDN). Significantly different from control; * $p < 0.05$; ** $p < 0.01$.

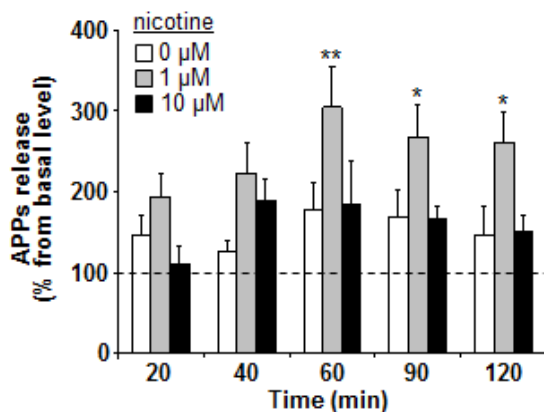


Figure 2: Release of APPs from hippocampal slice preparations *in vitro* in the presence of different concentrations of nicotine in the incubation medium up to a 2 h treatment time. The data are represented as a percentage change from basal level at the zero time point (broken line) and are expressed as mean+SEM ($n = 6$ rats per group). Significantly different from control (0 μM); * $p < 0.05$, ** $p < 0.01$.

Co-treatment of hippocampal slices with the non subunit-selective nicotinic receptor antagonist mecamylamine (10 μM) was used in order to confirm that the nicotine-associated increase in APPs was a receptor-mediated event. The antagonist significantly attenuated the nicotine-induced release of APPs ($F[1, 16] = 20.56, p < 0.001$) (Figure 3). Similarly, EGTA (2.5 mM), a preferential Ca^{2+} chelator, also showed similar reduction in APPs release ($F[1, 16] = 8.50, p = 0.01$) (Figure 3) suggesting a possible role of calcium ions in the cellular process regulating nicotine-induced APPs release from the hippocampus. Treatment with mecamylamine or EGTA alone had no effect on the basal protein release.

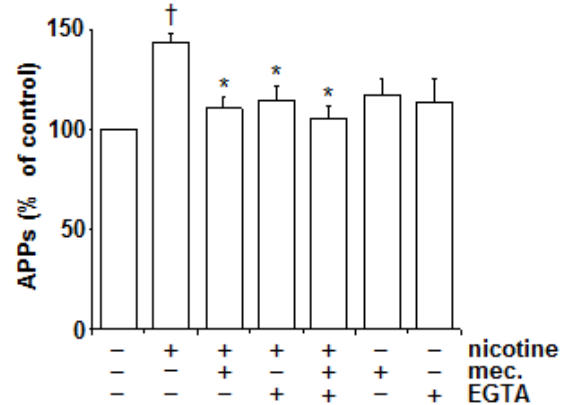


Figure 3: The effect of the nicotinic AChR antagonist mecamylamine (mec., 10 μM) or the preferential calcium ion chelator EGTA (2.5 mM) on nicotine-induced APPs release from hippocampal slice preparations as a percentage change from control value taken as 100%. All data are shown as mean+SEM ($n = 6$ animals per group). † $p < 0.05$ from control group; * $p < 0.05$ from nicotine-alone group.

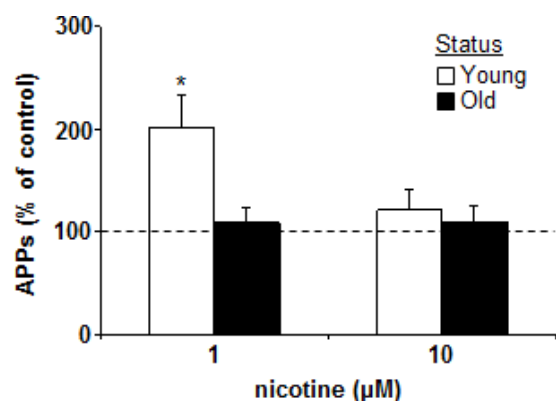


Figure 4: Nicotine-induced APPs release from hippocampal slice preparations generated from young (6-8 week, $n = 5$ per group) and old (19-21 months, $n = 5$ per group) rats following a 1 h incubation period. The quantity of secreted protein was expressed as a percentage of control value without nicotine treatment in the same experiment (broken line). Data is expressed as mean+SEM. Significantly different from control; * $p < 0.05$.

Age-dependent effects on the release of APPs from hippocampal tissue was tested using hippocampal slice preparations derived from young and old rat brains and incubated in different concentrations of nicotine. Two-way ANOVA revealed a nicotine dose ($F[2, 24] = 5.12$, $p < 0.05$), age ($F[1, 24] = 5.72$, $p < 0.05$) and dose \times age ($F[2, 24] = 4.15$, $p < 0.05$) effect denoting that not only the nicotine concentration but also the age of the rats had a significant effect on the observed levels of APPs released (Figure 4).

3. Effects of nicotine on spatial learning

All treatment groups showed a general decrease in escape latency over training blocks and repeated measures ANOVA revealed significant effects of days ($F[3, 60] = 140$, $p < 0.001$) and blocks ($F[1, 20] = 54.3$, $p < 0.001$). The LDN group appeared to find the platform faster than controls over training days 1 and 2 whereas the HDN group appeared to take longer to find the platform especially on days 2 and 4 of training (Figure 5A). There was a significant group \times day interaction ($F[6, 60] = 2.45$, $p < 0.05$) and further analysis of each day revealed that the groups only differed significantly on day 4 ($F[2, 22] = 4.37$, $p < 0.05$). Post-hoc pairwise comparisons confirmed that the HDN but not the LDN rats took longer than controls ($p < 0.05$). Analysis of the swim speed data (Figure 5B) revealed that there was no significant group \times day interaction suggesting that the group difference on the latency measure was not due to changes in swim speed.

Analysis of the percentage time spent in each quadrant during the probe trial suggested that while control and LDN rats showed a preference for the quadrant where the platform was located during training, the HDN rats did not (data not shown). Statistical analysis revealed a significant quadrant \times treatment interaction ($F[5, 60] = 3.22$, $p = 0.05$) and subsequent analysis of each group showed a highly significant quadrant effect in controls ($F[2, 33] = 24.3$, $p < 0.001$) and LDN ($F[2, 15] = 7.96$, $p < 0.05$) but not HDN ($F[2, 12] = 1.85$, $p > 0.1$). Analysis of the training quadrant only confirmed a significant effect of group ($F[2, 20] = 3.93$, $p < 0.05$) with only HDN rats spending significantly less time than controls in the region where the platform had been located ($p < 0.05$). Statistical analysis of the annulus crossings also revealed a highly significant quadrant by treatment interaction ($F[6, 60] = 2.6$, $p < 0.05$) and post-hoc analyses of each group confirmed a similar pattern with only the HDN group failing to show a significant bias for the platform location.

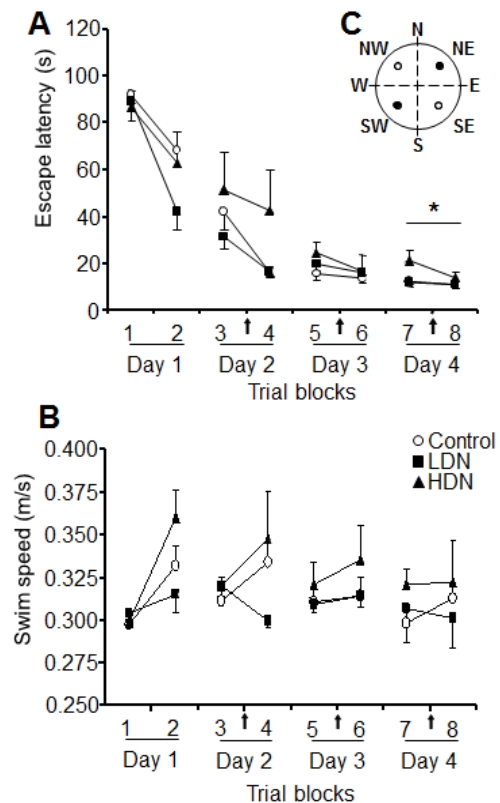


Figure 5: The effect of chronic nicotine infusions on the acquisition of a spatial task in the Morris water-maze. Escape latency values (A) in rats receiving the high dose nicotine infusion (HDN, $n = 5$) were significantly higher during day 4 of training compared to that of control rats (Control, $n = 12$) and low dose nicotine (LDN, $n = 6$). Swim speed values (B) were not significantly different among the groups for day 4 or any other training day. Results are shown as mean \pm SEM (* $p < 0.05$ for HDN compared to the control group by Dunnett's test). Arrows on x-axis indicate when BrdU was administered. (C) Diagrammatic representation of the pool quadrants with the two possible platform locations (NE and SW, closed circles) and the eight possible start locations.

4. Effects of nicotine neural cell genesis

Spatial learning increased the number of BrdU-labelled cells in the dentate gyrus in all groups relative to rats not receiving the task (Figure 6). Administration of the higher nicotine dose appeared to reduce BrdU-labelled cells both in trained and non-trained animals. Statistical analysis revealed both a significant task ($F[1, 40] = 26.87$, $p < 0.001$) and treatment ($F[2, 40] = 20.44$, $p < 0.001$) effect but no interaction between the two factors ($F[2, 40] = 1.19$, $p > 0.1$). Post-hoc analysis of the treatment effect confirmed that only the high dose of nicotine reduced the number of cells produced compared to controls ($p < 0.05$).

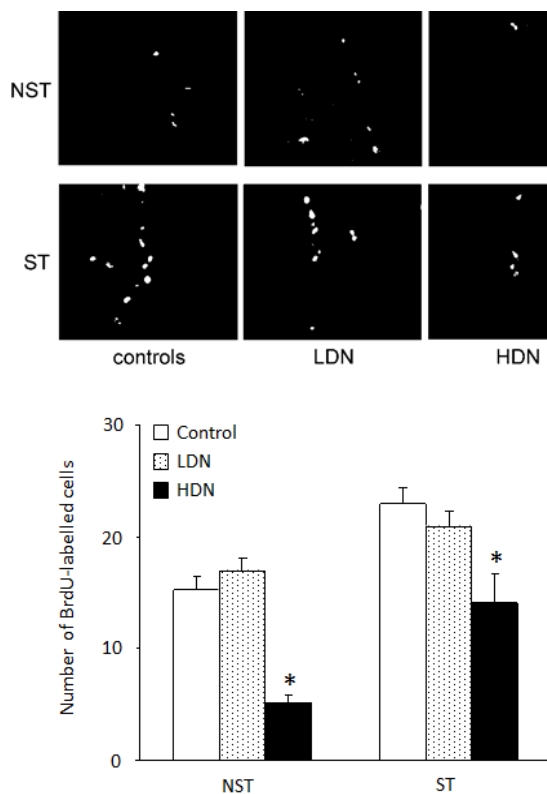


Figure 6: The effect of chronic nicotine infusions on neural cell genesis in the dentate gyrus in the presence (ST) and absence (NST) of spatial learning task. Exposure to the spatial learning task significantly increased the number of BrdU-labelled cells. High-dose nicotine (HDN, $n = 5$) infusion, but not the low dose (LDN, $n = 6$) reduced the number of BrdU cells in both trained and untrained rats compared to control ($n = 12$). Results are shown as mean+SEM (* $p < 0.05$ compared to control by Dunnett's test). Inset above: Representative black and white photomicrographs in a section of the dentate gyrus showing cells labelled for BrdU (Scale bar: 50 μ m).

Discussion

Previous studies investigating the effects of nicotine on APP metabolism were mostly carried out using *in vitro* methods.^{22,24,30,31} The present data have shown that, *in vivo*, sustained exposure to nicotine evokes an increase in particulate APP in the hippocampus. This response to nicotine was regionally-selective to the extent that it was not observed in the other brain regions investigated and age-dependent in that it was not observed in aged rats. *In vitro* studies with hippocampal tissue derived from young rats showed that exposure to the low (1 μ M) concentration of nicotine increased the release of APPs, an effect that was attenuated by prior administration of a nicotinic receptor antagonist. The results suggest that acute stimulation of neuronal nicotinic receptors stimulates the release of APPs through a nicotinic-receptor mechanism and that, in the hippocampus, chronic exposure to nicotine

may result in a compensatory increase in APP formation. This conclusion is consistent with the results of previous studies which have shown that nicotine stimulates APP expression and secretion in various cell lines.^{22,24,30}

The effects of nicotine on APPs release from hippocampal slices were attenuated markedly by the removal of Ca^{2+} from the incubation medium suggesting that the observed effects of nicotine on APPs release depend upon an increase in the intracellular free Ca^{2+} concentration putatively caused by depolarisation of the cells. Previously it was reported that activation of nAChRs, particularly the $\alpha 7$ subtype which shows high Ca^{2+} permeability, increases the intracellular Ca^{2+} levels leading to increased α -secretase levels via PKC and ERK activation.³²

In vivo effects of nicotine treatment on APPp were restricted to the hippocampus. The reason for this regionally-selective response remains unclear. However, the hippocampus is one of the few brain regions which continue to exhibit neurogenesis postnatally. This process persists in the rat up to approximately one year in age but then decreases markedly as the animal age further.³³ The neurogenesis is promoted by exposure to neurotrophic factors including APPs.³⁴ Hippocampal neurogenesis is also initially increased in the hippocampus of transgenic mice manipulated to over-express APP.³⁵ This effect has been interpreted as compensatory response to the early pathology of the condition.^{35,36}

There is evidence that APPs may have significant neuroprotective and neurotrophic properties³⁷ and it has been suggested that the increases in APPs release evoked by nicotine may mediate the proposed neuroprotective properties of nicotine and contribute to its ability to maintain cognitive function in a manner that might be therapeutically valuable.³⁸ The data presented here, however, cast some doubt on this hypothesis because the effect of nicotine on APPs release was not observed in aged rats or hippocampal tissue taken from aged animals. Thus, it would seem that the effects of nicotine on APP processing might be a response observed in younger animals only. If this were to translate to human brain, it implies that nicotine may be of only limited value in the treatment of AD or other age-related neurodegenerative conditions.

The results presented here have also shown that the constant infusion of the higher dose of nicotine tested evoked a modest impairment of the acquisition of a

spatial learning task and significantly impairs retention of spatial memory when tested with a probe trial. The effects of nicotine in this task were influenced by the nicotine dose used, the deficits only being observed in animals treated with the higher dose. While the reason for the differences in response to the two doses tested remains to be established, it may be significant to note that the infusion of nicotine at a rate of 4 mg/kg/day causes desensitisation of the population of neuronal nicotine receptors that mediate the effects of nicotine on dopamine release in the nucleus accumbens and dorsal striatum as well as noradrenaline release.³⁹ By contrast, infusions of the lower dose tested (0.25 mg/kg/day) does not desensitise these receptors and, indeed, results in sensitisation of this response in animals subsequently challenged acutely with a single dose of nicotine.³⁹ Thus, the constant infusion of nicotine at the two doses investigated here have already been shown to exert differential effects in the brain in a manner which suggests that they interact differentially at the diverse populations of neuronal nicotinic receptor that mediate the psychopharmacological responses to the drug. The results suggest that the deficit in spatial memory observed in the presented study may only be detected in animals in which many of the neuronal nicotinic receptors are desensitised by the drug.

Many of the studies that have explored the effects of nicotine on learning and memory have employed intermittent injections of the drug administered briefly before each test. This data suggests that the constant infusion of nicotine, at a dose that causes desensitisation of many neuronal nicotinic receptors, has different effects on learning when compared with those reported previously using intermittent injection of the drug at doses (e.g. 0.3 mg/kg s.c.) that generate similar plasma levels.⁴⁰ Other studies have also shown that intermittent injections of nicotine stimulate the mesolimbic and nigrostriatal dopamine pathways and the noradrenergic projections to the hippocampus whereas concomitant infusion of nicotine blocks these responses.⁴¹ Thus, for at least three neural systems within the brain, the effects of infused and intermittently injected nicotine are quite different.

The constant infusion of the higher dose of nicotine also inhibited BrdU incorporation into cells in the dentate gyrus whereas the lower dose did not have a significant effect. The experimental design used for this study did not differentiate between putative effects on cell genesis and survival. However, Abrous and colleagues⁴² also reported that a reduction in cell genesis in the dentate gyrus of animals that self-administered nicotine was only significant for the rats that self-administered the higher doses (0.04 or 0.06 mg/kg/infusion) of the drug, and did not occur in the subventricular zone of the hippocampus.

By contrast, the constant infusion of nicotine into the mouse decreased BrdU incorporation into the granule cell layer of the olfactory bulb but had no significant effect in the dentate gyrus.⁴³ The reason for the difference in the effects remains unclear, although the conditions of the experiment and the species studied were clearly different.

Training in the water-maze task was associated with a significant increase in neural cell genesis in this area of the brain. Dobrossy et al⁴⁴ have suggested that increased cell genesis in the dentate gyrus is a feature of the later stages of water-maze learning. However, Dobrossy and colleagues⁴⁴ used a single 4 trial training block per day whereas in this study 2 x 4 trial training blocks per day were used. Therefore it seems reasonable to assume that fewer days will be required for the animals to reach the later phase of learning, a conclusion consistent with the evidence that the animals approached asymptotic performance by day 3 of training. It is tempting to suggest that the deficits in learning, seen most clearly in the probe trial, evoked by the constant infusion of nicotine may be related directly to its effects on cell genesis in the hippocampus. However, this conclusion should be treated with some caution since nicotine suppressed the incorporation of BrdU in both control and maze-trained animals. Therefore, although the absolute level of cell genesis in the rats trained in the maze with nicotine was lower than that found in saline-treated rats trained in the maze, cell genesis in both the saline- and nicotine-treated animals appeared to be elevated to approximately the same extent by training.

In conclusion, the results reported have shown that nicotine administration influences the APP metabolism in the hippocampus of young, but not aged rats. The mechanisms which mediate this response have not been elucidated fully but appear to depend upon stimulation of the nAChR and the availability of intracellular free Ca^{2+} . The results provide some support for the hypothesis that nicotinic receptor agonists may have some value in slowing the progress of AD pathology although this must be tempered by the evidence that the response to nicotine may be age-dependent. Furthermore, chronic infusion of nicotine at a dose that is likely to cause desensitisation of many of the neuronal nicotinic receptors in the brain reduces neural cell genesis and impairs acquisition of spatial memory. Further studies on the effects of nicotine and similar compounds in animal models of AD may provide further insights into the potential therapeutic value of these compounds.

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