Psychopharmacology (2006) 184: 540–546
DOI 10.1007/s00213-005-0086-4

ORIGINAL INVESTIGATION

Charles Scerri · Caroline A. Stewart · Kieran C. Breen · David J. K. Balfour

The effects of chronic nicotine on spatial learning and bromodeoxyuridine incorporation into the dentate gyrus of the rat

Received: 30 December 2004 / Accepted: 27 May 2005 / Published online: 16 July 2005

Abstract *Rationale:* Nicotine is reported to improve learning and memory in experimental animals. Improved learning and memory has also been related to increased neurogenesis in the dentate gyrus (DG) of the hippocampal formation. Surprisingly, recent studies suggest that self-administered nicotine depresses cell proliferation in the DG. *Objective:* To test the hypothesis that the effects of nicotine on cell proliferation in the DG and learning and memory depend upon the nicotine dose administered. *Methods:* Rats were chronically infused from subcutaneous osmotic mini pumps with nicotine (0.25 or 4 mg kg^{-1} day^{-1}) or the saline vehicle for 10 days. Half the rats in each treatment group were trained to locate a hidden platform in a water maze task on days 4–7; a probe trial was performed on day 8. The remaining rats remained in their home cages. The effects of nicotine and of training in the water maze task on cell genesis in the DG were determined by measuring 5-bromo-2′-deoxyuridine (BrdU) uptake using fluorescence immunohistochemistry. *Results:* Training in the water maze task increased cell proliferation in the DG. Infusions of nicotine at 4 mg kg^{-1} day^{-1}, but not 0.25 mg kg^{-1} day^{-1}, decreased cell proliferation in both untrained animals and animals trained in the maze and impaired spatial learning. *Conclusions:* The data suggest that learning in the water maze task is impaired by higher doses of nicotine tested, and that this response may be related to reduced cell genesis in the DG.

C. Scerri · C. A. Stewart · K. C. Breen · D. J. K. Balfour (✉)
Section of Psychiatry and Behavioural Sciences,
Division of Pathology and Neuroscience,
Ninewells Hospital and Medical School,
University of Dundee,
Dundee, Scotland, DD1 9SY, UK
e-mail: d.j.k.balfour@dundee.ac.uk
Tel.: +44-1382-632524
Fax: +44-1382-633923

C. Scerri
Department of Clinical Pharmacology and Therapeutics,
University of Malta,
Msida, MSD 06, Malta

Keywords Nicotine · Bromodeoxyuridine · Memory · Cognition · Water maze · Hippocampus

Introduction

There is evidence from both clinical and preclinical studies that nicotine elicits improvements in cognitive function (Warburton and Rusted 1993; Hahn et al. 2002; Levin and Rezvani 2002), and it has been suggested that these effects contribute to the reinforcing properties of the drug sought by smokers (Heishman 1999). The effects on cognitive function are thought to be related to stimulation of neurotransmitter systems within areas of the brain that are important for cognitive processing (Levin and Simon 1998; Singer et al. 2004). 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 Alzheimer's disease (Changeux et al. 1998; Picciotto and Zoli 2002).

The hippocampus is thought to play a pivotal role in learning and memory, particularly in spatial learning (Morris et al. 1982; Jacobs and Schenk 2003). The subgranular zone of the dentate gyrus (DG) within the hippocampal formation is one of the few areas of the brain in which neurogenesis continues to occur into adulthood (Altman 1962; Eriksson et al. 1998). Increased neurogenesis can be produced by a variety of treatments, including an enriched environment (Brown et al. 2003), physical activity (van Praag et al. 1999) and antidepressant drugs (Malberg et al. 2000; Santarelli et al. 2003). Neurogenesis has also been specifically implicated in learning tasks that involve the hippocampus. If rats are trained on a hippocampal-dependent associative task, the survival of newborn cells is increased (Gould et al. 1999), whereas performance is impaired by an inhibition of cell proliferation (Shors et al. 2001). It is therefore surprising that a recent study found that self-administered nicotine decreased neurogenesis in this area of the brain (Abrous et al. 2002), a response that appears inconsistent with the reported cognitive-enhancing properties of nicotine.

This study investigated the effects of constantly infused nicotine on spatial learning in the Morris water maze and cell proliferation in the DG. Two doses of nicotine were selected to permit a comparison of the effects of nicotine over doses that have some relevance to the tobacco smoking habit but exert different effects at the receptor level. The high dose chosen (4 mg kg^{-1} day^{-1}) results in blood nicotine concentrations (approximately 80 ng/ml) that would only be found in heavy smokers (Benwell et al. 1995) and desensitises many of the neuronal nicotinic receptors in the brain through which nicotine exerts its effects (Benwell et al. 1995; Pidoplichko et al. 1997). The lower dose (0.25 mg kg^{-1} day^{-1}) maintains a blood nicotine concentration (approximately 8 ng/ml) commonly found in the plasma of light smokers and does not desensitise these receptors (Benwell et al. 1995).

Materials and methods

Subjects

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-controlled (21°C) and humidity-controlled (50±10%) environment on a 12-h light/dark cycle, with lights on at 6:00 am. Food and water were provided ad libitum. All the experiments were conducted during the light phase of the cycle and were in accordance with UK Home Office regulations and covered by Home Office project licence number 60/2845.

Drug treatment

Rats were divided into three groups: a control group that received saline, a 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) (Table 1). All reagents were purchased from commercially available sources unless otherwise indicated. Nicotine or saline was administered subcutaneously via osmotic mini pump. (−)-Nicotine hydrogen tartrate (Sigma, UK) was dissolved in 0.9% saline solution. The doses of nicotine were calculated as those of the free base. Before filling the osmotic mini pumps, drug solutions and vehicle were sterilised by filtration through a 20-μm filter.

Osmotic mini pumps (Alzet, ALZA Corporation, Palo Alto, CA, USA) were filled with drug or vehicle as instructed by the manufacturer. They were implanted subcutaneously (s.c.) in the flank under inhalational anaesthesia (5% halothane for induction, 3% for maintenance) through a small incision on the back at the level of the shoulders on day 1 of the experiment. After a 2-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 learning).

Spatial learning task

Rats were trained in an open-field water maze (Morris 1984) that was 1.8 m in diameter and 0.6 m deep filled to 30 cm with water (26±2°C). The water was made opaque by the addition of reconstituted milk powder, and various extra maze cues were illuminated from below the level of the maze using floodlights. Rats were trained for 4 days to find an escape platform (10 cm diameter) hidden 2 cm below the surface of the water. For half of the rats the platform was located in the north-east quadrant, and for the other half, in the south-west quadrant (see Fig. 1c). Every day, rats in the ST groups were given two blocks of training composed of four trials each. The two blocks of training were separated by a 3-h interval. Eight different starting points were used in a pseudorandom order. A maximum search time of 120 s was allowed for each trial. If the rats found the platform during this period, they were left on the platform for 30 s before beginning the next trial. If any rat failed to find the platform, it was placed on the platform and again left for 30 s. On the day following the last day of training, a probe trial was conducted to assess retention of the platform location where the platform was removed and each rat was allowed to swim for 60 s. The rats were tracked using an overhead video camera. The analogue signal was digitised (Hawk V10, Wild Vision) by computer (Acorn A5000) to allow offline analysis (WaterMaze software, University of Edinburgh). Performance

Table 1 Experimental protocol

Drug Treatment	Days 4–7	Days 5–7	Day 8	Day 10
Saline	NST	BrdU	Home cage	Transcardial perfusion
Saline	ST	BrdU	Probe trial	Transcardial perfusion
Nicotine (0.25 mg kg^{-1} day^{-1})	NST	BrdU	Home cage	Transcardial perfusion
Nicotine (0.25 mg kg^{-1} day^{-1})	ST	BrdU	Probe trial	Transcardial perfusion
Nicotine (4 mg kg^{-1} day^{-1})	NST	BrdU	Home cage	Transcardial perfusion
Nicotine (4 mg kg^{-1} day^{-1})	ST	BrdU	Probe trial	Transcardial perfusion

Mini pumps containing nicotine or its saline vehicle were inserted on day 1 of the experiment. The animals trained in the water maze task (ST) following the procedure described in the Materials and methods section. Untrained controls (NST groups) remained in their home cages. On day 8, a probe trial was performed in the animals trained on the spatial learning task. The animals were transcardially perfused on day 10 and the brains removed for processing

during task acquisition was assessed from escape latencies (time taken to find the platform). Swim speeds were calculated by dividing the distance travelled on each trial by the 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 bromodeoxyuridine incorporation

Cells formed from dividing progenitors were identified using bromodeoxyuridine (BrdU) (Sigma, UK), which integrates into DNA during the S phase of DNA synthesis (Cooper-Kuhn and Kuhn 2002). 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 (Table 1). These 3 days were selected for the injections because the primary objective of the experiment was to explore putative changes in cell proliferation that occurred during the period that the animals were learning the spatial task. NST rats were injected at identical time points. On day 10 of the experiment, the rats were deeply anaesthetised with an overdose of pentobarbital sodium (i.p.) and perfused transcardially with 40 ml of saline followed by 140 ml of ice-cold paraformaldehyde (4% in 0.2 M sodium phosphate buffer, pH 7.4). The brain was removed and postfixed in paraformaldehyde solution for at least 24 h. Coronal sections (20 μm) were cut throughout the hippocampus using a cryostat. Every eighth section was thaw-mounted on slides.

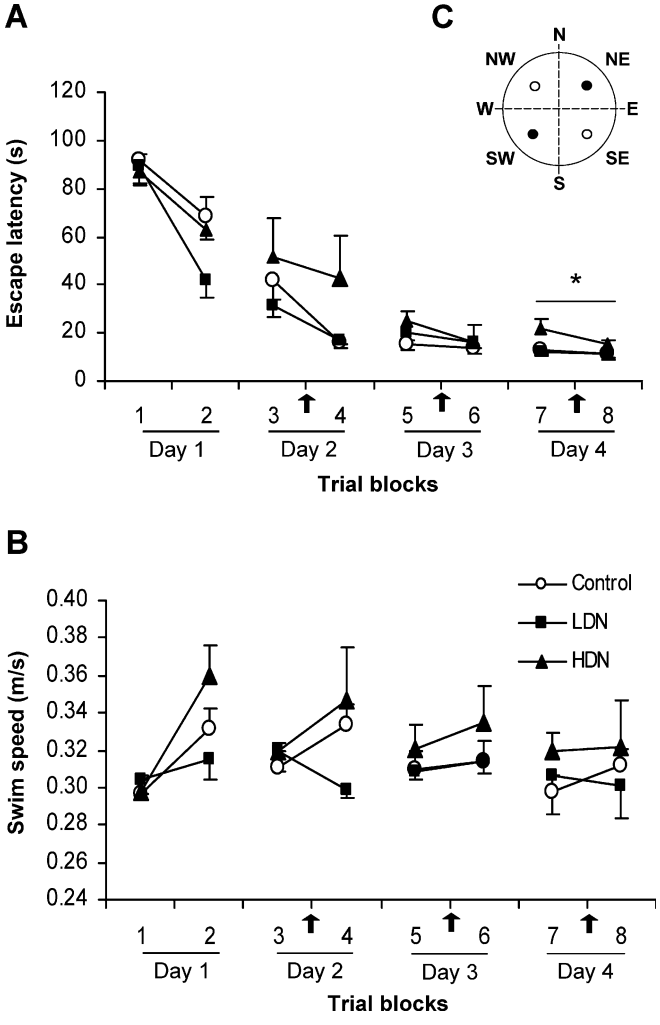

Fig. 1 The effect of chronic nicotine infusions on the acquisition of a spatial navigation task in the water maze. Escape latency values (**a**) in rats receiving the high-dose nicotine (*HDN*, *n*=5) infusion were significantly higher during day 4 of training compared to that of control (*Control*, *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 + SEM (*P<0.05 for HDN compared to the control group by Dunnett's *t* 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

Immunohistochemical techniques were used to identify the cells that had incorporated BrdU during cell division. A 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. To maximise antigen retrieval the following pretreatment steps were followed: DNA denaturation was performed by incubating the slides in 0.01 M citric acid (pH 6.0, 100°C, 10 min), followed by membrane permeabilisation (0.01% trypsin in 0.1 M Tris/0.02 M $CaCl_2$, 10 min) and acidification (2 M HCl, 25°C, 30 min). The non-specific binding of primary antibodies was blocked by incubating the slides in the blocking buffer, phosphate-buffered saline (PBS) containing 0.25% Triton X-100 and 10% normal horse serum (PBST), for 1 h. The slides were then exposed to primary antibodies: mouse-anti-NeuN (1:50; Chemicon International, UK) and rat-anti-BrdU (1:200; Harlan Sera-Lab, UK) in PBST for 3 days at 4°C. Sections were then washed in PBS for 10 min, blocked in PBST for 30 min and incubated with secondary antibodies [fluorescein isothiocyanate (FITC)-labelled anti-mouse immunoglobulin G (IgG), Scottish Antibody Production Unit (SAPU); 1:50 and tetramethylrhodamine isothiocyanate (TRITC)-labelled anti-rat IgG, SAPU; 1:160] for 1 h at room temperature. The slides were then washed in PBS for 10 min, and after adding a few drops of Vectorshield (Vector Laboratories, UK), coverslips were placed on the slides and sealed with clear nail varnish.

Slides were coded before counting to ensure objectivity. BrdU-labelled cells were visualised using a fluorescent microscope (Zeiss Axioskop II). Alternate sections were selected for analysis. Hence, eight hippocampal sections per rat were taken at intervals of 320 μm. All BrdU-labelled cells within the granule cell layer and hilus of the DG were counted, and the number of BrdU-labelled cells for each subject was expressed as a mean per section (Madsen et al. 2000).

Data analysis

All data were analysed using the Statistical Package for Social Sciences (SPSS, version 11.5), and the level of statistical significance was taken as $P<0.05$. Analysis of variance (ANOVA) with repeated measures was used to determine group differences in behavioural performance, both during the acquisition of the spatial learning task and during the probe trial. The percentage time spent in each training quadrant was not independent, and therefore, the numerator degree of freedom was reduced by one. Post hoc analysis was carried out using further ANOVA or Dunnett's multiple comparisons. Group differences in BrdU-labelled cells in the DG were analysed using two-way ANOVA and post hoc using Dunnett's *t* tests.

Results

Effect of nicotine on the spatial learning task

A preliminary inspection of individual acquisition curves revealed that one rat in the HDN group did not improve significantly over the eight blocks of trials. The mean latency for this rat was more than two standard deviations from the group mean on the last block of training, and the results for this rat were therefore removed prior to analysis of the data. 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 control animals 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 (Fig. 1a). There was a significant group by 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 (Dunnett's *t*, $P<0.05$). The decrease in escape latency over the early days in the LDN group did not reach statistical significance. Analysis of the swim speed data (Fig. 1b) revealed that there was no significant group by day interaction [$F<1$], 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 C 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

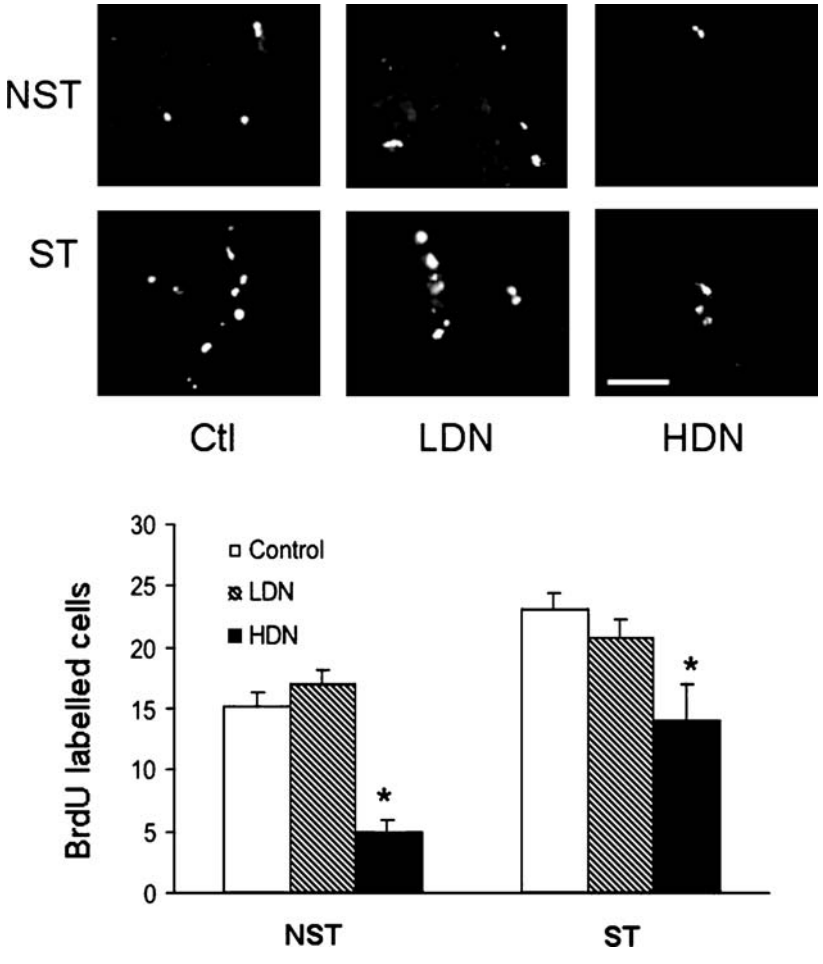

Fig. 2 The influence of chronic nicotine and spatial learning on the number of BrdU-labelled cells in the DG. High-dose nicotine (*HDN*, *n*=5) infusion, but not the low-dose (*LDN*, *n*=6), reduced the number of BrdU cells in both trained (*ST*) and untrained (*NST*) rats compared to control (*Ctl*, *n*=12). Exposure to the spatial learning task significantly increased the number of BrdU-labelled cells independently of treatment. Results are shown as mean + SEM (*$P<0.05$ compared to control by Dunnett's *t* test). *Inset above*: Representative *black and white* photomicrographs in a section of the DG showing cells labelled for BrdU (*Scale bar* 50 μm)

by treatment interaction [$F(5,60)=3.22$, $P=0.05$], and subsequent analysis of each group showed a highly significant quadrant effect in the control group [$F(2,33)=24.3$, $P<0.001$] and LDN group [$F(2,15)=7.96$, $P<0.05$] but not the HDN group [$F(2,12)=1.85$, $P>0.1$]. Analysis of the training quadrant alone 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 (Dunnett's t, $P<0.05$). 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.

Effect of nicotine infusion on BrdU incorporation

Spatial learning increased the number of BrdU-labelled cells in the DG in all groups relative to rats not receiving the task (Fig. 2). 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 ($P<0.05$).

Discussion

This study has shown that the constant infusion of the higher dose of nicotine (4 mg kg^{-1} day^{-1}) evoked a modest impairment of the acquisition of a spatial learning task and significantly impaired retention of spatial memory when tested with a probe trial. This contradicts other studies that have reported nicotine-induced enhancements of cognitive function in experimental models such as the radial maze and water maze tasks (Decker et al. 1992; Socci et al. 1995; Levin and Torry 1996; Levin and Rezvani 2000; Rezvani and Levin 2001; Levin 2002; Hahn et al. 2002). By contrast, there have been reports that nicotine can also cause impairments of learning and memory in these models (Dunnett and Martel 1990) or have no effect (Hagan et al. 1989; Attaway et al. 1999). Furthermore, a recent study reported that heavy smoking resulted in impaired cognitive function measured in mid-life (Richards et al. 2003). Thus, the response to nicotine seems to depend critically upon factors such as the test employed, the dose of the drug administered and the route of administration.

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. Indeed, during the early phase of training, the lower dose of nicotine tested tended to enhance the acquisition of the task, although this did not achieve statistical significance. While the reason for the differences in response to the two doses tested remains to be established, other studies in this laboratory have also revealed differential effects of the drug when it was infused at 0.25 or 4 mg kg^{-1} day^{-1}. Specifically, the infusion of nicotine at a rate of 4 mg kg^{-1} day^{-1} caused desensitisation of the population of neuronal nicotinic receptors that mediate the effects of nicotine on dopamine release in the nucleus accumbens and dorsal striatum as well as noradrenaline release in the hippocampus (Benwell et al. 1995). By contrast, infusions of the lower dose tested (0.25 mg kg^{-1} day^{-1}) did not desensitise these receptors and, indeed, resulted in sensitisation of this response in animals subsequently challenged acutely with a single dose of nicotine (Benwell et al. 1995). Inasmuch as it has been investigated, intermediary doses of the drug also caused desensitisation of neuronal nicotinic receptors in the rat brain (Benwell et al. 1995; Pidoplichko et al. 1997; Balfour et al. 2000). Thus, the constant infusion of nicotine at the two doses investigated here have been shown to exert differential effects in the brain in a manner which suggests that they interact differentially with the diverse populations of neuronal nicotinic receptor that mediate the psychopharmacological responses to the drug. It can be hypothesised that the deficit in spatial memory observed in the present study may only be detected in animals in which many of the neuronal nicotinic receptors are desensitised.

Many studies that have demonstrated beneficial effects of nicotine on learning have employed intermittent injections of the drug administered briefly before each test (Decker et al. 1992; Socci et al. 1995; Curzon et al. 1996; Hahn et al. 2002). This route of administration can be expected to stimulate the nicotinic receptors through which nicotine exerts its effects in the brain, whereas the infusions are more likely to result in desensitisation. This conclusion is supported by the fact that intermittent injections of nicotine stimulate the mesolimbic and nigrostriatal dopaminergic pathways and the noradrenergic projections to the hippocampus, whereas concomitant infusion of nicotine blocks these responses (Benwell and Balfour 1997). This study suggests that the constant infusion of nicotine at a dose that generates similar plasma levels to intermittent injections (e.g. ~0.3 mg/kg s.c.) (Turner 1975) has different effects on learning because its predominant effect is the desensitisation of neuronal nicotinic receptors.

The constant infusion of the higher dose of nicotine also inhibited BrdU incorporation into cells in the DG, whereas the lower dose did not have a significant effect. Abrous et al. (2002) also reported that the self-administration of high doses of nicotine (0.04 or 0.06 mg kg^{-1} infusion^{-1}) reduced cell proliferation in the DG. This effect of self-administered nicotine was restricted to the DG and did not occur in the subventicular zone. By contrast, in mice, the constant infusion of nicotine decreased BrdU incorporation into the granule cell layer of the olfactory bulb, but had no significant effect in the DG (Mechawar et al. 2004). 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 BrdU incorporation into the DG. A recent study has suggested that increased cell proliferation in the DG is a feature of the later stages of water maze learning (Dobrossy et al. 2003). However, Dobrossy et al. (2003) used a single four-trial training block per day, whereas in this study, 2×4 trial training blocks per day were used. Therefore, it seems reasonable to assume that fewer days would be required for the animals to reach the later phase of learning, as supported by the observation that the animals approached asymptotic performance by day 3 of training. It is tempting to suggest that the nicotine-induced deficits in learning, evidenced by impaired performance in the probe trial, may be causally related to its effects on cell proliferation in the hippocampus. However, this conclusion should be treated with caution, since nicotine suppressed the incorporation of BrdU in both untrained and maze-trained animals. Thus, although maze-trained animals displayed a lower level of BrdU incorporation when treated with nicotine as opposed to saline, cell genesis in both saline- and nicotine-treated animals appeared to be elevated to approximately the same extent by training.

There is evidence that cell proliferation in the hippocampus may also be enhanced by increased motor activity (van Praag et al. 1999). It is possible that changes in BrdU incorporation into the DG in rats trained in the maze could be caused by the increase in motor activity associated with daily training. This is unlikely to provide a complete explanation for the data, since Dobrossy et al. (2003) demonstrated that yoked rats, allowed to swim in the pool for the same time as rats trained to locate an escape platform, did not show the increase in neurogenesis observed in the trained animals.

Nicotine, when administered at high doses, is reported to have anxiogenic properties (File et al. 1998; File et al. 2000). Stressful stimuli are also reported to inhibit neurogenesis in the hippocampus (Gould et al. 1997). Thus, it is possible that the effects of the higher dose of nicotine on BrdU incorporation in the DG could reflect anxiogenic effects of nicotine. However, studies that demonstrated an anxiogenic response to nicotine employed subcutaneous injections of the drug. In the present investigation, nicotine given by slow infusion from a subcutaneous mini pump avoids the high peak in nicotine evoked by subcutaneous injections of the drug. Furthermore, previous studies in our laboratory have shown that nicotine infused at 4 mg kg^{-1} day^{-1} did not elicit an anxiogenic response in the elevated plus maze (Benwell et al. 1994). The same dose had no significant effects on the plasma corticosterone concentration in control, unstressed animals or the raised corticosterone levels found in animals exposed acutely to the elevated plus-maze test (Benwell et al. 1994). These results imply that the chronic administration of nicotine by this route does not, in itself, evoke a stress response, nor does it influence the response to an anxiogenic stimulus, such as the plus-maze.

In conclusion, this study has shown that the constant infusion of nicotine at a dose that is likely to cause desensitisation of many of the neuronal nicotinic receptors in the brain reduces BrdU incorporation into the DG and impairs acquisition of a water maze task. However, the nicotine infusions did not exert a selective effect on BrdU incorporation in the rats tested in the water maze. As a result, further studies are required before reaching a conclusion regarding a causal relationship between reduced BrdU incorporation and impaired spatial learning and alternative behavioural sequelae cannot be excluded.

Acknowledgements This study was partly funded by a research grant awarded by the University of Malta.

References

Abrous DN, Adriani W, Montaron MF, Aurousseau C, Rougon G, Le Moal M, Piazza PV (2002) Nicotine self-administration impairs hippocampal plasticity. J Neurosci 22:3656–3662

Altman J (1962) Are new neurons formed in the brains of adult mammals? Science 135:1127–1128

Attaway CM, Compton DM, Turner MD (1999) The effects of nicotine on learning and memory: a neuropsychological assessment in young and senescent Fischer 344 rats. Physiol Behav 67:421–431

Balfour DJK, Wright AE, Benwell MEM, Birrell CE (2000) The putative role of extra-synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. Behav Brain Res 113: 73–83

Benwell MEM, Balfour DJK (1997) Regional variation in the effects of nicotine on catecholamine overflow in rat brain. Eur J Pharmacol 325:13–20

Benwell MEM, Balfour DJK, Khadra LF (1994) Studies on the influence of nicotine infusions on mesolimbic dopamine and locomotor responses to nicotine. Clin Invest 72:233–239

Benwell MEM, Balfour DJK, Birrell CE (1995) Desensitization of the nicotine-induced mesolimbic dopamine responses during constant infusion with nicotine. Br J Pharmacol 114:454–460

Brown J, Cooper-Kuhn CM, Kempermann G, van Praag H, Winkler J, Gage FH, Kuhn HG (2003) Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. Eur J Neurosci 17:2042–2046

Changeux JP, Bertrand D, Corringer PJ, Dehaene S, Edelstein S, Lena C, Le Novere N, Marubio L, Picciotto M, Zoli M (1998) Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. Brain Res Brain Res Rev 26:198–216

Cooper-Kuhn CM, Kuhn HG (2002) Is it all DNA repair? Methodological considerations for detecting neurogenesis in the adult brain. Brain Res Dev Brain Res 134:13–21

Curzon P, Brioni JD, Decker MW (1996) Effect of intraventricular injections of dihydro-beta-erythroidine (DH beta E) on spatial memory in the rat. Brain Res 714:185–191

Decker MW, Majchrzak MJ, Anderson DJ (1992) Effects of nicotine on spatial memory deficits in rats with septal lesions. Brain Res 572:281–285

Dobrossy MD, Drapeau E, Aurousseau C, Le Moal M, Piazza PV, Abrous DN (2003) Differential effects of learning on neurogenesis: learning increases or decreases the number of newly born cells depending on their birth date. Mol Psychiatry 8:974–982

Dunnett SB, Martel FL (1990) Proactive interference effects on short-term memory in rats: I. Basic parameters and drug effects. Behav Neurosci 104:655–665

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4:1313–1317

File SE, Kenny PJ, Ouagazzal AM (1998) Bimodal modulation by nicotine of anxiety in the social interaction test: role of the dorsal hippocampus. Behav Neurosci 112:1423–1429

File SE, Kenny PJ, Cheeta S (2000) The role of the dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety. Pharmacol Biochem Behav 66:65–72

Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J Neurosci 17:2492–2498

Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ (1999) Learning enhances adult neurogenesis in the hippocampal formation. Nat Neurosci 2:260–265

Hagan JJ, Jansen JH, Broekkamp CL (1989) Hemicholinium-3 impairs spatial learning and the deficit is reversed by cholinomimetics. Psychopharmacology 98:347–356

Hahn B, Shoaib M, Stolerman IP (2002) Nicotine-induced enhancement of attention in the five-choice serial reaction time task: the influence of task demands. Psychopharmacology 162:129–137

Heishman SJ (1999) Behavioral and cognitive effects of smoking: relationship to nicotine addiction. Nicotine Tob Res 1(Suppl 2): S143–S147

Jacobs LF, Schenk F (2003) Unpacking the cognitive map: the parallel map theory of hippocampal function. Psychol Rev 110: 285–315

Levin ED (2002) Nicotinic receptor subtypes and cognitive function. J Neurobiol 53:633–640

Levin ED, Rezvani AH (2000) Development of nicotinic drug therapy for cognitive disorders. Eur J Pharmacol 393:141–146

Levin ED, Rezvani AH (2002) Nicotinic treatment for cognitive dysfunction. Curr Drug Targets CNS Neurol Disord 1:423–431

Levin ED, Simon BB (1998) Nicotinic acetylcholine involvement in cognitive function in animals. Psychopharmacology 138:217–230

Levin ED, Torry D (1996) Acute and chronic nicotine effects on working memory in aged rats. Psychopharmacology 123:88–97

Madsen TM, Treschow A, Bengzon J, Bolwig TG, Lindvall O, Tingstrom A (2000) Increased neurogenesis in a model of electroconvulsive therapy. Biol Psychiatry 47:1043–1049

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104–9110

Mechawar N, Saghatelyan A, Grailhe R, Scoriels L, Gheusi G, Gabellec MM, Lledo PM, Changeux JP (2004) Nicotinic receptors regulate the survival of newborn neurons in the adult olfactory bulb. Proc Natl Acad Sci U S A 101:9822–9826

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11:47–60

Morris RGM, Garrud P, Rawlins JNP, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297:681–683

Picciotto MR, Zoli M (2002) Nicotinic receptors in aging and dementia. J Neurobiol 53:641–655

Pidoplichko VI, DeBiasi M, Williams JT, Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. Nature 390:401–404

Rezvani AH, Levin ED (2001) Cognitive effects of nicotine. Biol Psychiatry 49:258–267

Richards M, Jarvis MJ, Thompson N, Wadsworth ME (2003) Cigarette smoking and cognitive decline in midlife: evidence from a prospective birth cohort study. Am J Public Health 93:994–998

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805–809

Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E (2001) Neurogenesis in the adult is involved in the formation of trace memories. Nature 410:372–376

Singer S, Rossi S, Verzosa S, Hashim A, Lonow R, Cooper T, Sershen H, Lajtha A (2004) Nicotine-induced changes in neurotransmitter levels in brain areas associated with cognitive function. Neurochem Res 29:1779–1792

Socci DJ, Sanberg PR, Arendash GW (1995) Nicotine enhances Morris water maze performance of young and aged rats. Neurobiol Aging 16:857–860

Turner DM (1975) Influence of route of administration on metabolism of [14C]nicotine in four species. Xenobiotica 5:553–561

van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266–270

Warburton DM, Rusted JM (1993) Cholinergic control of cognitive resources. Neuropsychobiology 28:43–46