Nicotine blocks stress-induced impairment of spatial memory and long-term potentiation of the hippocampal CA1 region

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Abstract
The effect of chronic nicotine treatment on chronic psychosocial stress-induced impairment of short-term memory and long-term potentiation (LTP) was determined. An ‘intruder’ stress model was used to induce psychosocial stress for 4–6 wk, during which rats were injected with saline or nicotine (1 mg/kg s.c.) twice a day. The radial arm water maze memory task was used to test hippocampus-dependent spatial memory. Chronic psychosocial stress impaired short-term memory without affecting the learning phase or long-term memory. Concurrent chronic nicotine treatment prevented stress-induced short-term memory impairment. In normal rats chronic nicotine treatment had no effect on learning and memory. Extracellular recordings from the CA1 region of anaesthetized rats showed severe reduction of LTP magnitude in stressed rats, which was normalized in nicotine-treated stressed rats. Nicotine had no effect on LTP in control animals. These results showed that chronic nicotine treatment improved hippocampus-dependent spatial memory and LTP only when impaired by stress.

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Introduction

Nicotine reduces memory impairment associated with ageing (White and Levin, 2004), brain lesions (Decker et al., 1992; Levin et al., 1993b), and other brain disorders including Alzheimer’s disease (White and Levin, 1999; Wilson et al., 1995), Parkinson’s disease (Maggio et al., 1998), schizophrenia (Levin et al., 1996b), and attention deficit hyperactivity disorder (Conners et al., 1996). Moreover, the incidence of Alzheimer’s and Parkinson’s diseases is reported to be lower among smokers than non-smokers (Fratiglioni and Wang, 2000). Additionally, nicotine is reported to improve working-memory performance of normal rats in the radial-arm maze (Levin et al., 1993a) and facilitates the induction of long-term potentiation (LTP) in hippocampal slices (Fuji et al., 1999). The effects of nicotine on memory and LTP are prevented by treatment with the non-selective nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine (Fuji et al., 1999; Levin et al., 1987).

Although LTP is not the only mechanism involved in memory formation, it is widely accepted as a cellular correlate for the encoding of memory. A vast number of pharmacological and electrophysiological experiments indicate a close correlation between LTP and memory (see Lynch, 2004; Martin et al., 2000 for review). However, experiments in mutant animals have generated some controversy in the LTP–memory relationship (e.g. Hinds et al., 1998; Silva et al., 1992). On the other hand, activation of alternative pathways that compensate for the knocked-out gene transcription could explain at least some of the controversial results (Grant et al., 1992; Hinds et al., 1998).

Although adaptation to stress may be a necessary mechanism for survival, high-intensity or long-duration stress impairs the function of certain brain structures including the hippocampus (McEwen, 2000). Chronic psychosocial stress has been shown to impair hippocampus-dependent memory (Baran et al., 2005; Gerges et al., 2004b) and block LTP in the CA1 region, but not in the dentate gyrus (DG) region of the hippocampus (Gerges et al., 2001). In addition, stress...
alters the basal levels of key signalling molecules involved in memory and LTP (Gerges et al., 2004a). These effects are believed to be initiated by enhanced glutamate and glucocorticoid secretion (Magarinos and McEwen, 1995; Watanabe et al., 1992). Glucocorticoid receptors exist at high density in the hippocampus, which is also enriched with α1 and α2 nAChR subtypes (Fabian-Fine et al., 2001; Wada et al., 1989). High glucocorticoid levels activate Type-II glucocorticoid receptors, which leads to excitotoxicity and hippocampal atrophy (McEwen, 1994). The reported increase in nicotine consumption rate among habitual and occasional smokers (Boos and Croft, 2004) during stress is, perhaps, a sign of ‘self-medication’ to counter the detrimental effect of stress-induced high corticosteroid levels on memory and LTP.

In this report we utilized two approaches, electrophysiological and behavioural, to determine the effect of daily nicotine treatment on chronic psychosocial stress-induced impairment of memory and LTP.

**Materials and methods**

Adult male Wistar rats (200–250 g; Harlan, Indianapolis, IN, USA) were housed six per cage and had free access to food and water in a 12 h light/dark cycle at 25 °C. All procedures involving animals were carried out in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals and with the approval of the University of Houston Institutional Animal Care and Use Committee.

**Treatments**

There were four treatment groups in this study: normal (control), stressed (stress), nicotine-treated normal (nicotine) and nicotine-treated stressed (nicotine/stress) rats. The control and stress groups received saline (0.9% w/v NaCl) s.c. twice a day for 4–6 wk. The nicotine and nicotine/stress groups were treated with nicotine (Sigma, St. Louis, MO, USA); 1 mg/kg s.c. twice a day for 4–6 wk.

**Induction of psychosocial stress**

The stress and nicotine/stress groups were stressed using a form of ‘intruder’ psychosocial model described previously (Alkadhi et al., In Press; Gerges et al., 2001). Briefly, after allowing rats of each group to remain with the same cage mates for at least 1 wk to establish social hierarchy, stress was developed by daily random switching of two animals from one cage to the other, for a period of 4–6 wk. This psychosocial stress model is known to produce stress by disrupting the established social hierarchy, such that rats must continuously adjust to new situations. Psychosocial stress was marked by an increase in serum corticosterone levels (Gerges et al., 2001) and elevation of blood pressure (Alkadhi et al., In Press).

**The radial arm water maze procedure**

After 4 wk of stress and/or nicotine treatment, rats were tested for performance in the radial arm water maze. The maze consisted of a water-filled black circular tub with six V-shaped stainless steel structures arranged to form a swimming field of an open central area and six arms (Diamond et al., 1999; Gerges et al., 2004b). The water temperature was maintained at 25 °C. One of the arms, the goal arm, contained a black platform submerged 1 cm below the water level. Starting from an arm (any arm other than the goal arm), the rat must find the submerged platform by swimming to the end of the goal arm. Rats were allowed 1 min per trial to find the submerged platform. A trial ended when the rat located the submerged platform within 1 min, where it was allowed a 15 s stay on the platform before removal to begin the next trial in a different start arm. A trial also ended if the rat did not locate the platform by the conclusion of a 1-min period, at which point the experimenter would guide the rat to the goal arm and allow it to stay on the platform for 15 s before removal to begin the next trial. A correct selection occurred when the rat swam to the goal arm, whereas error was registered when the rat entered an arm other than the goal arm. Each rat was allowed four consecutive trials (acquisition phase) followed 15 min later by a short-term memory trial, and then 2-h and a 24-h long-term memory trials, with different start arms on each trial. For the memory tests, the procedure of counting errors was the same as in the acquisition phase trials except the rats were not allowed any time on the platform upon arrival and were never guided by the experimenter. The hidden platform was placed in the same goal arm for all seven trials for a given rat on a given day, and randomly placed in any one of the six arms across days, with the exception that the hidden platform was never in the same arm on two consecutive days. The maze experiments were done in a dimly lit room and rats had to use cues in the room to spatially memorize the location of the platform on that day. Trials were performed every day for a period of 6 d or more, until rats reached days to criterion (DTC). To reach the DTC, a rat needed to make not more than a total of one error on the memory test trial for three consecutive days of testing.
Electrophysiological procedures

Standard procedures for in-vivo recording from the CA1 and DG regions of anaesthetized rats were performed as described (Gorges et al., 2001). At the end of treatment, rats were anaesthetized with urethane (1.2 g/kg i.p., Sigma) and placed in a stereotaxic frame (nose bar at 0.0). Then, the skull was exposed and holes were drilled in predetermined places (see below) to access the left CA3, right CA1/DG and angular bundle regions. A concentric bipolar electrode was placed in the CA3 region of the left hippocampus at an angle of 5° towards the midline to stimulate the Schaffer collateral/commissural pathway (AP-3, L 3.5, D 2.8). A second bipolar stimulating electrode (twisted Teflon-coated stainless-steel wires) was placed in the right angular bundle (AP-8, L 4.4, D 3). A recording electrode (tip resistance 1–5 MΩ) filled with 1% Fast Green dye (to disclose the position of the electrode tip) in 2 M NaCl, was placed in the stratum pyramidale of the CA1 region or the DG region of the right hippocampus (AP-3, L 1.8, D 2.0/3.0). First, the left CA3 region was stimulated (square wave pulse, 0.3 ms duration) and responses were recorded from the right CA1 region. After finishing stimulation/recording of the Schaffer collateral pathway/CA1 region, the recording electrode was moved down by 1 mm into the DG region. The angular bundle was then stimulated (square wave pulse, 0.3 ms duration) to record from the DG region. A train of 20 pulses at 100 Hz was applied to either Schaffer collateral or angular bundle to evoke LTP in the CA1 or DG region respectively. A stimulus intensity of ~30–40% of the maximum response was chosen to evoke test responses. This intensity was found to cause maximum enhancement of field excitatory post-synaptic potential (fEPSP) slope (Gorges et al., 2001). Evoked population spikes (pSpikes) were amplified (Axoclamp 2A amplifier, Axon Instruments Inc., Foster City, CA, USA) and recorded from the CA1 or DG of the right hippocampus. The slope of fEPSP, a measure of the intensity of synaptic activity, and amplitude of pSpike, a measure of the number of neurons reaching threshold, were quantified as described (Gorges et al., 2001). Computer-based stimulation, data acquisition and recording were accomplished by the use of pCLAMP8.2 software and DigiData 1322A (Axon Instruments Inc.).

Statistical methods

Statistical differences among various groups were evaluated using one-way ANOVA, followed by Tukey’s post-test; p < 0.05 was considered statistically significant. Data are expressed as means ± S.E.M.

Results

Hippocampus-dependent spatial memory

Exposure to psychosocial stress has been reported to cause spatial memory impairment (Gorges et al., 2004b). Rats in all groups were tested in the radial arm water maze to measure the hippocampus-dependent spatial memory, which is a hippocampus-dependent function (Morris et al., 1982).

Chronic nicotine treatment prevents stress-induced spatial memory impairment

The within-day learning and memory tasks showed that chronic stress impaired only the short-term memory (trial 5) without affecting the long-term memory (trials 6 and 7) or acquisition (learning) phase (trials 1–4; Figure 1). On days 1 and 2 of the radial arm water maze task, there was no significant difference in the number of errors made by rats in the control, nicotine, stress and nicotine/stress groups in all trials, including short- and long-term memory trials (Figure 1a).

On days 3–6 (Figure 1b, c) all groups learned the within-day location of the platform equally well, as determined by the marked reduction of errors in trials 2–4 of these particular days indicating that stress did not affect the learning process. However, during these days of testing the short-term memory was significantly impaired in the stress group (trial 5). This was indicated by the observation that the stress group made significantly (p < 0.05) more errors in short-term memory (trial 5) to locate the hidden platform than the control and nicotine groups. In the nicotine/stress group, rats made significantly (p < 0.05) fewer errors in locating the hidden platform than the untreated stress rat group indicating that chronic nicotine treatment prevented the effect of stress on short-term memory. Furthermore, there was no significant difference in the number of errors made among nicotine/stress, control and nicotine groups in the short-term memory trials. Long-term memory was not affected by stress or nicotine treatment (Figure 1b, c). Additionally, chronic nicotine treatment in normal rats had no effect on learning, short-term memory, or long-term memory, compared to the untreated control group (Figure 1) suggesting a restorative/protective rather than a promotive effect of nicotine.

To further confirm the results, we also recorded the number of days a rat needed to reach a performance criterion (DTC). In agreement with Gorges et al. (2004b), this test showed that stress markedly impaired short-term memory (Figure 2b), as indicated by...
the higher number of days required by the stress group to reach DTC. Chronic nicotine treatment prevented stress-induced short-term memory impairment (Figure 2b). In the nicotine/stress group rats required significantly ($p < 0.05$) fewer days to reach DTC than the untreated stress rats (Figure 2b). There was no significant difference among nicotine/stress, control and nicotine groups in the short-term memory trial. In addition, there was no significant difference among all groups in the DTC of long-term memory (Figure 2c, d) and learning phase (Figure 2a).

**Electrophysiological results**

We have also assessed the effect of nicotine and/or stress on the expression of LTP, the widely accepted cellular correlate of learning and memory, in anaesthetized rats. We recorded the evoked potential, pSpike, from the CA1 or DG region of the rat hippocampus before and after induction of LTP in the control and experimental groups.

**The CA1 region: effects of high-frequency stimulation (HFS)**

Chronic nicotine treatment and/or stress did not affect basal synaptic transmission in the CA1 region as indicated by input/output (I/O) curves constructed at the beginning of every experiment (data not shown). In the control and nicotine groups, HFS (20 pulses at 100 Hz) caused an increase in the slope of fEPSP and amplitude of pSpike lasting more than 60 min in the CA1 region of the hippocampus (Figure 3a, b). The fEPSP slope (Figure 3a) of the control and nicotine groups, measured 1 h after HFS, was $127 \pm 10\%$ and $125 \pm 12\%$ of baseline respectively. The amplitude of...
pSpike (Figure 3b) was 219 ± 22% and 247 ± 41% respectively. No difference in LTP magnitude between the control and nicotine groups was observed in the CA1 region. Thus, nicotine alone seems to have no effect on the normal magnitude of LTP (Figure 3).

The CA1 region: chronic nicotine treatment prevents stress-induced impairment of LTP

In agreement with a previous study (Gerges et al., 2001), chronic stress suppressed HFS-evoked LTP in the CA1 region. The fEPSP slope (93 ± 8% of baseline, Figure 3a) and pSpike amplitude (115 ± 20%, Figure 3b) in the stress group, measured 1 h after HFS, were significantly (p < 0.05) lower than those of the control and nicotine groups (Figure 3a). In the nicotine/stress group, chronic nicotine treatment completely inhibited stress-induced impairment of LTP in the CA1 region. One hour after HFS, the fEPSP slope (127 ± 13%, Figure 3a) and pSpike amplitude (206 ± 21%, Figure 3b) in the nicotine/stress group were significantly (p < 0.05) different from those of the stress group, but not from those of control and nicotine groups. These results suggest that chronic nicotine treatment prevents stress-induced LTP suppression in the CA1 region of the hippocampus.

The DG region: effects of HFS

As in the CA1 region, basal synaptic transmission was unchanged in the nicotine, stress and stress/nicotine groups in the DG region. Following HFS, LTP was expressed as an increase in the slope of fEPSP (114 ± 2% of baseline, Figure 4a) and pSpike amplitude (144 ± 18%, 1 h after HFS, Figure 4b) in control rats. The fEPSP slope (Figure 4a) and pSpike amplitude (Figure 4b) of the nicotine group were similar to those of the control group (113 ± 2% and 142 ± 6% respectively).

Neither psychosocial stress, nor nicotine treatment seems to change LTP in the DG (Figure 4a, b). The fEPSP slope (Figure 4a) and pSpike amplitude (Figure 4b) in all groups, measured 1 h after HFS, were similar to those of control and higher than the baseline.
values at any given time. Thus, in agreement with Gerges et al. (2001), stress does not seem to impair LTP in the DG region.

**Discussion**

Marked impairment of hippocampus-dependent memory and suppression of LTP in the CA1 region of the hippocampus have been observed during stress (Gerges et al., 2001, 2004b). The principal finding of this study is that chronic nicotine treatment completely prevented stress-induced LTP suppression. Each point is the mean ± S.E.M. from 4–7 rats. Insets are representative pSpikes.

Figure 3. High-frequency stimulation (HFS; 20 pulses at 100 Hz applied at time zero) of the Schaffer collateral pathway evoked LTP of the hippocampal CA1 region. LTP is measured as increases in slope of fEPSP (a) and amplitude of pSpike (b) in urethane-anesthetized Wistar rats. Chronic nicotine treatment completely prevented stress-induced LTP suppression. Each point is the mean ± S.E.M. from 4–7 rats. Insets are representative pSpikes.

Figure 4. Hippocampal LTP evoked by high-frequency stimulation (HFS; 20 pulses at 100 Hz applied at time zero) of the angular bundle pathway of the DG region and measured as increase in the slope of fEPSP (a) and amplitude of pSpike (b) in urethane-anesthetized Wistar rats. All points from all groups are higher than the baseline value (paired t test), but not significantly different from each other (ANOVA, followed by Tukey test). Each point is the mean ± S.E.M. from 4–7 rats. Insets are representative pSpikes.

nicotine attenuates memory deficits associated with a variety of conditions that negatively impact learning and memory including ageing, brain lesions, Alzheimer’s disease, Parkinson’s disease, schizophrenia, and attention deficit hyperactivity disorder (White and Levin, 1999; Wilson et al., 1995). However, reported effects of nicotine on normal memory have been variable. While some investigations show memory enhancement after nicotine treatment (Levin et al., 1993a; Wesnes and Warburton, 1984), others show no effect (Dunne et al., 1986; Parrott and Winder, 1989), or even memory impairment (Park et al., 2000; Sorensen et al., 1991). The inconsistent results may be the consequence of variations in nicotine dose, treatment...
Nicotine blocks stress-induced impairment of spatial memory and LTP

The present results show that nicotine does not enhance normal memory or LTP magnitude in the CA1 and DG regions. In contrast to nicotine, chronic psychosocial stress impairs short-term memory and inhibits hippocampal LTP in the CA1 but not in the DG region, which confirms earlier reports (Gerges et al., 2001, 2004b). The mechanism by which stress causes impairment of memory and LTP is not well understood, however, increased release of glutamate and glucocorticoids has been suggested as a possible mechanism (Margarinos and McEwen, 1995; Watanabe et al., 1992). Both the high-affinity (Type-I) mineralocorticoid and low-affinity (Type-II) glucocorticoid receptors exist on hippocampal neurons (Beck et al., 1994; Pavlides et al., 1996). In normal physiological conditions, the basal levels of glucocorticoids, which activate Type-I receptors, are necessary for memory (Conrad et al., 1999) and LTP (Pavlides et al., 1996). However, under stressful conditions, high glucocorticoid concentration secreted from the adrenal cortex suppresses LTP (Pavlides et al., 1996) and impairs memory (Conrad et al., 1999) by activating Type-II receptors. Further, increased release of glucocorticoids and glutamate during stress have been shown to induce excitotoxicity, neuronal death and hippocampal atrophy (Margarinos and McEwen, 1995; Watanabe et al., 1992). In fact, exogenous glucocorticoids such as corticosterone and cortisol, in concentrations similar to those circulating during stress, induce neuronal death, hippocampal atrophy and impaired memory (Endo et al., 1996; Kirschbaum et al., 1996) and LTP (Pavlides et al., 1996). The atrophy, which might be an adaptation mechanism to limit the increased excitatory input after exposure to stress (Sapolsky, 1999), has been shown to induce reversible impairment of learning and memory (Luine et al., 1994).

Chronic stress alters the levels of key molecules involved in memory and LTP. The basal levels of phosphorylated CaMKII (p-CaMKII) in the CA1 region of the hippocampus are reduced after chronic stress (Gerges et al., 2004a). Chronic stress also reduces the expression of BDNF mRNA in the hippocampus (Smith et al., 1995). On the other hand, the basal levels of protein phosphatases, such as calcineurin, are increased during stress (Gerges et al., 2004a). Overexpression of calcineurin in the hippocampus impairs memory (Mansuy et al., 1998) and inhibits LTP (Gerges et al., 2004a; Winder et al., 1998). Therefore, reduction of essential kinases and augmentation of protein phosphatases during stress could account for the impairment of LTP/memory observed in the CA1 region of stressed rats. In contrast to the effect on the CA1 region, chronic stress causes a decrease in calcineurin basal levels in the DG region, consequently allowing p-CaMKII levels to remain normal.
(Gerges et al., 2003). Normal levels of p-CaMKII may be responsible for the expression of normal LTP in the DG region in stressed rats reported in this and previous reports (Gerges et al., 2001).

In behavioural studies, two forms of spatial memory are described; working and reference memory. In the working memory, information is acquired and constantly updated, thus, it is a dynamic, trial-dependent, form of spatial memory (Diamond et al., 1996; Olton and Papas, 1979). This type of memory is impaired when hippocampal function is compromised (Boobot et al., 1998; Ohno et al., 1993; Steele and Morris, 1999). Reference memory in contrast, is a static, trial-independent, form of memory, in which the information acquired remains unchanged across many days of training. Numerous studies show that working, but not reference memory is impaired by stress (Diamond et al., 1996, 1999; Gerges et al., 2004b; Mizoguchi et al., 2001; Park et al., 2001; Radecki et al., 2005; Roskoden et al., 2005; Sandstrom, 2005; Sandstrom and Hart, 2005; Thomas et al., 1991; Woodson et al., 2003). Additionally, high doses of chronic nicotine (2.5 times the dose used in this study), show a significantly improved working memory, but not reference spatial memory during radial arm maze tasks in normal rats (Levin et al., 1993a, 1996a). The radial arm water maze memory task used in this report is basically a test for hippocampus-dependent working memory (Diamond et al., 1999; Gerges et al., 2004b).

We conclude that under our experimental conditions, nicotine has no effect on learning and memory in normal animals; however, in chronically stressed rats nicotine antagonizes the deleterious effect of stress on short-term memory and LTP. These results strengthen the view that nicotine works primarily in a need-based manner (i.e. when there is memory impairment). Therefore, nicotine under our experimental conditions seems to have a restorative, rather than a promotive effect on memory function.

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Statement of Interest

None.

References


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