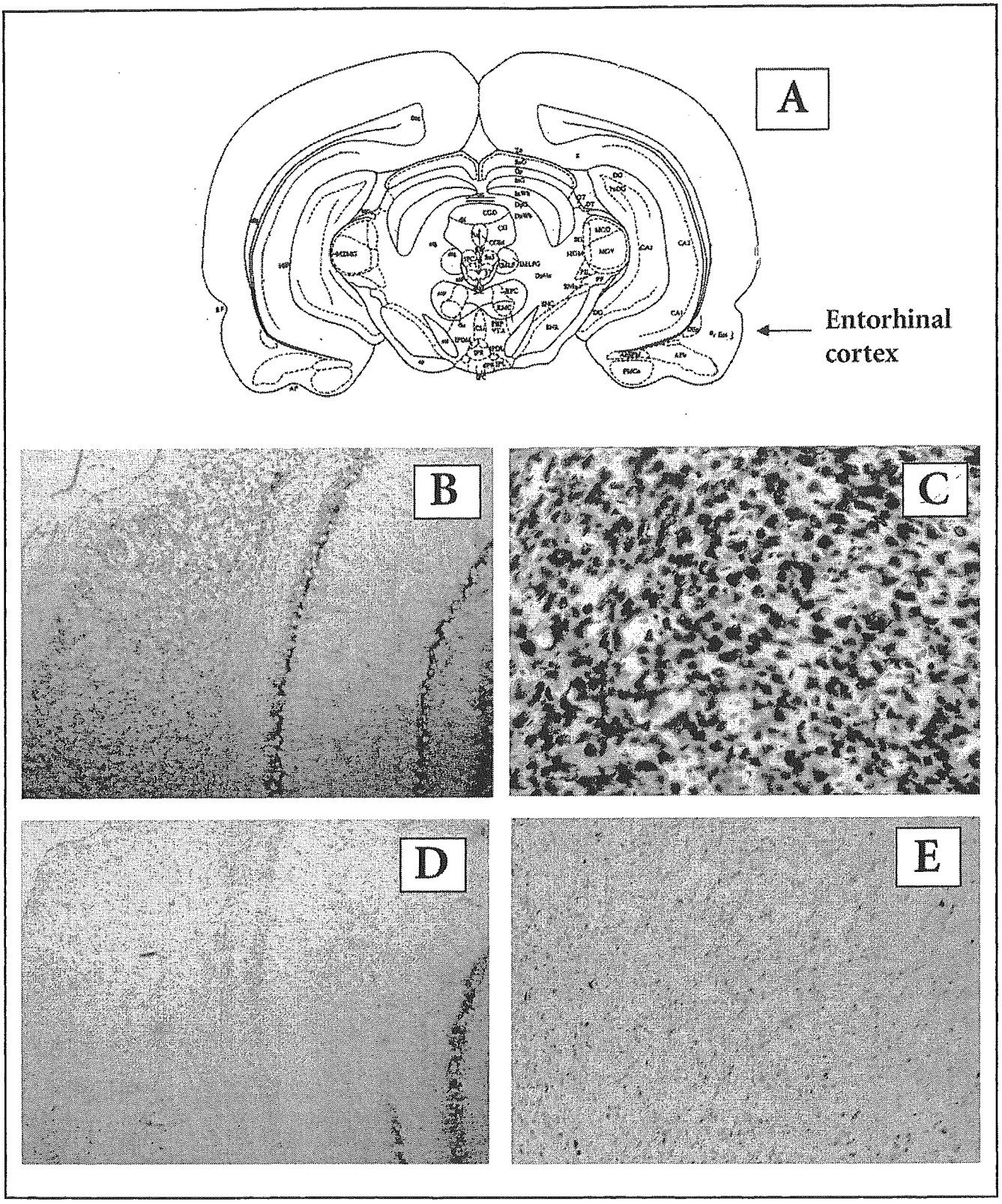

Figure 1.
(A): Schematic representation of a bilateral entorhinal cortex lesion.
Photomicrographs of coronal sections through the entorhinal cortex stained with Cresyl violet illustrating the extent of damage:
(B) Vehicle injection into the entorhinal cortex. (C) Enlarged view of that shown in (B).
(D) Ibotenic acid injection into the entorhinal cortex. (E) Enlarged view of that shown in (D).
The section was sampled from the injection sites where the maximal amount of cell damage was observed.

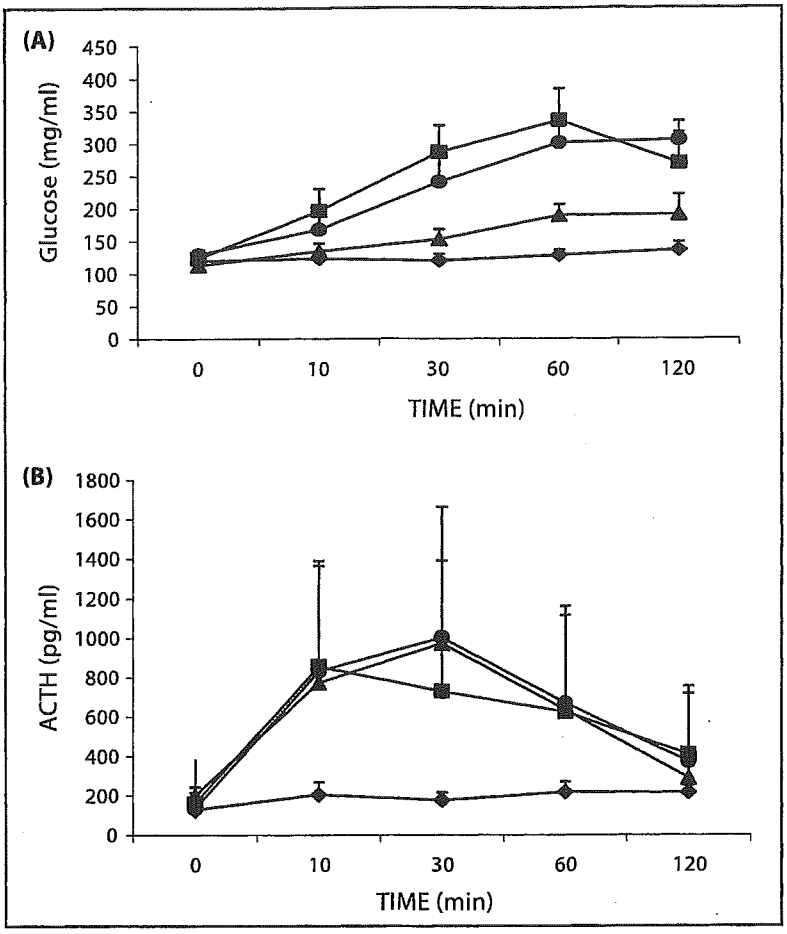

Figure 2.
Plasma ACTH and blood glucose concentrations in rats microinjected by neostigmine into the hippocampus.
(A) Blood glucose concentrations. Repeated ANOVA showed that entorhinal-lesioned rats had significantly lower concentrations than unlesioned rats ($p<0.0001$)
■, Group 1: unlesioned neostigmine-injected (n=6);
◆, Group 2: unlesioned saline-injected (n=5);
▲, Group 3: lesioned neostigmine-injected (n=11);
●, Group 4: sham-operated, neostigmine-injected (n=6).
(B) Plasma ACTH concentrations. Repeated ANOVA showed no significant difference between entorhinal lesioned rats and rats who had saline injected into the entorhinal cortex.
■, Group 1 unlesioned neostigmine-injected (n=6);
◆, Group 2: unlesioned saline-injected (n=5);
▲, Group 3: lesioned neostigmine-injected (n=6);
●, Group 4: sham-operated, neostigmine-injected (n=5).

tion of various mediators such as adrenal steroids, catecholamines, cytokines, and tissue mediators [13]. Stress-related signals in the central nervous system initially act upon the hypothalamus. From there, signals, which respond to stress stimuli in the central nervous system, reach the peripheral nervous system through several pathways. Two main ones are; (1) autonomic neurons and the adrenal medulla system, which release catecholamines; and (2) the hypothalamic-pitutitary-adrenal system, known as the HPA axis, which releases glucocorticoids. The secretion of adrenocortical glucocorticoids is driven by the release of ACTH from corticotropes in the anterior pituitary gland. Neurons in the PVN are the most potent structures capable of inducing ACTH release in response to stress through the release of corticotropin-releasing factor [24].

Microinjections of neostigmine into the hippocampus produce hyperglycemia associated with the secretion of plasma cathecholamines, which showed similarities to stress responses. Regarding the mechanism responsible for the neostigmine-induced elevation of plasma glucose, at least four pathways had been hypothesized; (1) secreted epinephrine may directly act on the hepatic release of glucose, (2) epinephrine may induce the release of glucagon, (3) direct neuronal control in the pancreas causes glucagon secretion, (4) direct innervation in the liver induces glucose release [6]. Our previous studies showed that ACTH release is accompanied by c-fos expression, a universal marker of neuronal activation, in the PVN of the hypothalamus [24]. We also found that the bed nucleus of the stria terminalis (BNST) is involved in the regulation of ACTH release in response to hippocampal neostigmine injection [4,23]. This structure receives inputs directly from the ventral hippocampus area and sends a heavy axonal projection into neuroendocrine cell regions of the PVN [5,23]. Although lesions of the BNST attenuated the elevation in ACTH, and c-fos expression in the PVN induced by hippocampal neostigmine injection, blood glucose elevation was not altered by the BNST lesions [23,24]. This suggests that glucose and ACTH are regulated differently within the brain, and that BNST is not involved in the glucose regulation pathways.

The entorhinal cortex occupies a key position in the limbic system, functioning as a relay station between the hippocampus and neocortex. The entorhinal cortex is a major gateway for sensory information into the hippocampal formation. In the current study, the entorhinal cortex lesion attenuated blood glucose elevation but did not affect the ACTH response. These results suggest that the entorhinal cortex plays role in blood glucose regulation and not in ACTH regulation when the hippocampal cholinergic system is activated.

We previously found that the entorhinal cortex is involved in the stress response to immobilization but

not to insulin-induced hypoglycaemia [22]. Lesions in this area attenuate ACTH release induced by immobilization but have no effect on the blood glucose response. These findings are not compatible with what we observed in this study using hippocampal neostigmine injections. Although the hippocampal neostigmine–injection model shows similarities to the stress responses in terms of ACTH and glucose profiles in the plasma, the model may activate a pathway in the brain different from what is activated in response to immobilization.

Recent evidence suggests that various stressors activate different regulatory pathways. According to Herman and Cullinan [5], stressors can be divided into two categories. One category is processive (emotional/ psychological) stressors, which activate cortical and limbic areas before the PVN is activated. Signals from multiple sensory modalities are processed in these structures prior to final elaboration of the stress response. The other group is systemic (physical) stressors, which directly threaten the survival and activate the PVN through the ascending catecholaminergic pathway from the brainstem. According to Herman and Cullinan's definition immobilization stress is processive stress. Since the brain pathways activated in the hippocampal neostigmine-injection model were different from the responses to immobilization, this model may show similarities to other types of stress responses. Further investigation should be performed to elucidate the mechanism responsible for this. In conclusion, the present study showed the role of entorhinal cortex in regulating stress response induced by microinjection of neostigmine and the relationship between the entorhinal cortex and hippocampus in stress responses.

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Dopamine D2R DNA transfer in dopamine D2 receptor-deficient mice: Effects on ethanol drinking

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Received 4 March 2004; accepted 9 October 2004

Abstract

Dopamine (DA) signals are transmitted via specific receptors including the D2 receptors (D2R). Previous studies have shown that D2R upregulation in the nucleus accumbens (NAc) attenuated alcohol consumption. We hypothesized that upregulation of D2R in the NAc would significantly influence alcohol drinking. We tested this hypothesis by determining the effect that D2R upregulation has on alcohol intake in genetically altered mice lacking D2Rs. After a steady baseline of drinking behavior was established for all mice, a null vector or a genetically modified adenoviral vector containing the rat D2R cDNA was infused into the NAc of wild-type (*Drd2+/+*), heterozygous (*Drd2+/−*), and receptor-deficient mice (*Drd2−/−*). Ethanol intake and preference were then determined using the two-bottle choice paradigm. Our results indicated that *Drd2+/+* mice treated with the D2R vector significantly attenuated (58 %) their ethanol intake as well as reduced preference. *Drd2+/−* and mutant mice showed a similar attenuation, although the change was not as marked (12 %) and did not last as long. In contrast, *Drd2−/−* mice treated with the D2R vector displayed a temporary but significant increase (46 %) in ethanol intake and preference (consumption). These results supported the notion that the D2R plays an important role in alcohol consumption in mice and suggest that a key threshold range of D2R levels is associated with elevated alcohol consumption. Significant deviations in D2R levels from this range could impact alcohol

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0024-3205/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2004.10.061

consumption, and could help to explain possible individual variations in alcohol response, metabolism, sensitivity and consumption.
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Keywords: Alcoholism; Addiction; Gene therapy; Associative learning

Introduction

Alcoholism is one of the most widespread diseases of modern times. While significant advances have been made at understanding the mechanism(s) of alcohol abuse and addiction, much remains unanswered. Brain dopaminergic receptor systems are thought to be important for the rewarding effects of alcohol (Koob et al., 1998; Samson and Harris, 1992), and it has been proposed to be one of the neurotransmitters that modulate the predisposition to alcohol abuse (Di Chiara et al., 1996; George et al., 1995; Li, 2000). Previous research has shown that the reinforcing effects of alcohol involve among others the projections of DA cells to the NAc (Koob et al., 1987). In particular the mesolimbocortical DA pathway arising from the ventral tegmental area (VTA) and innervating the ventral striatum and the NAc, plays a role in incentive motivational processes (Horvitz, 2000).

Several studies have reported that the D2R is involved in transmitting the DA mediated reinforcing effects of alcohol (McBride et al., 1993a; Nowak et al., 2000; Stefanini et al., 1992). Clinical studies have implicated the role of D2Rs in alcoholism (Guardia et al., 2000; Lu et al., 2001; Matsumoto et al., 2001; Volkow et al., 1996a) and chronic alcohol consumption is associated with significant reductions in D2R concentrations (Guardia et al., 2000; Tajuddin and Druse, 1996; Volkow et al., 1996b, 2002). This has led to the hypothesis that low levels of D2R may predispose subjects to alcohol and drug use as a mean to compensate for the decrease in activation of reward circuits activated by these receptors (Blum et al., 1996; Volkow et al., 1999a,b, 2002). Several animal studies have supported these findings and have reported that D2R antagonists enhanced ethanol self-administration in selectively bred alcohol-preferring P rats (Dyr et al., 1993; Levy et al., 1991). In addition, it has been demonstrated that inbred ethanol preferring rats displayed lower D2R binding versus non-preferring rats (McBride et al., 1993a,b; Stefanini et al., 1992; Thanos et al., 2004). Recently it was demonstrated that D2Rs in the NAc participated in coding for a specific type of neural response to incentive contingencies and associative or spatial learning (Tran et al., 2002). Specifically, disruption of the D2R in the mesolimbocortical DA pathway changed neural responses to incentive stimuli in *Drd2−/−* mice. The strong bias to place-related neural activity in NAc neurons in the *Drd2−/−* mice suggested that neural substrates for spatial mapping of the environment were compromised in mice lacking the D2R (Tran et al., 2002).

DNA transfer (adenoviral) of the D2R into the NAc of adult Sprague Dawley rats, previously trained to self-administer alcohol, produced marked reductions in alcohol preference and consumption, which normalized after D2R gene expression returned to baseline levels (Thanos et al., 2001). Similarly, inbred ethanol preferring P rats treated with the D2R vector (in the NAc) significantly attenuated their alcohol preference (37 % decrease) and intake (48 % decrease), and these measures returned to pretreatment levels by day 20 (Thanos et al., 2004). In addition, chronic (7 weeks) ethanol exposure in P and NP rats

demonstrated a comparable D2R profile (Thanos et al., 2004) than was previously observed in naïve P and NP rats (McBride et al., 1993a).

Transgenic and gene knockout models including *Drd2−/−* mice have provided further insight into the genetically mediated differences in susceptibility to ethanol's effects, aiding our understanding of the genetic mechanism of alcohol abuse and alcoholism (Palmer et al., 2003). Specifically, *Drd2−/−* mice have been shown to have decreased preference and sensitivity to ethanol when compared with *Drd2+/+* (Phillips et al., 1998) but show a greater susceptibility to the development of locomotor sensitization with repeated ethanol treatment(Palmer et al., 2003). While these data provided powerful evidence of the involvement of the D2R in ethanol consumption, interpretation of the results requires further study, since *Drd2−/−* mice have a total absence of D2R throughout the brain; which may influence ethanol drinking in a different manner than localized D2R upregulation or downregulation. To start with, the absence of D2R will impede the animal's ability to learn an association between alcohol-induced increases in DA and the responses that are triggered by D2R activation. Also the potential role of altering local D2R concentrations on brain circuits involved with the rewarding responses to alcohol needs to be examined in these animals. Therefore, it is essential to study the effects of local D2R restitution on the sensitivity to alcohol in these *Drd2−/−* mice.

The purpose of this study was to determine the effects of manipulating D2R number in the NAc by DNA transfer techniques using a replication-deficient adenoviral vector containing cDNA coding for the D2R (AdCMV.D2R) on alcohol consumption in *Drd2−/−* mice compared to *Drd2+/−* and *Drd2+/+* mice. DNA transfer via adenoviral vector has been an effective strategy to introduce particular genes into tissue and has provided a high specificity targeting and delivery (Crystal, 1992; Suhr and Gage, 1993). The effectiveness of the present vector for intracerebral transfer of D2R as well as the expression of functional D2R effects has been previously well established (Ikari et al., 1995, 1999; Ingram et al., 1998; Ogawa et al., 2000; Thanos et al., 2001; Umegaki et al., 1997). Rats infected with this adenoviral vector produced significant increases in D2R expression with significant decreases in alcohol preference and intake (Thanos et al., 2001, 2004).

We hypothesized that upregulation of D2R in the NAc (by treatment with the D2R vector), would influence ethanol intake and preference depending on the animal's D2R status. More specifically we predicted that ethanol intake and preference would increase in the receptor-deficient mice receiving vector plus cDNA sequences and decrease in *Drd2+/−* and *Drd2+/+* mice.

Materials and methods

All studies were conducted in accordance with the guidelines established by the National Institutes of Health in *The Guide for Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Brookhaven National Laboratory. Mice were individually housed in a room controlled for temperature and humidity as well as a 12-hour light/dark (lights off 0700h) cycle. Food, water and ethanol were provided ad libitum.

Adult male congenic (N10) on C57Bl/6J mice were used in this study [WT ($Drd2^{+/+}$) HT ($Drd2^{+/-}$) and KO ($Drd2^{-/-}$)] and had a mean weight of 35 ± 4 g. The present study consisted of two groups: 1) D2R vector treated and 2) Null vector treated. Each group consisted of 10 mice per strain (*Drd2+/+*, *Drd2+/−* and *Drd2−/−*).

Microsurgery and vector microinfusion

Mice were anesthetized with an equal mixture of ketamine / xylazine (100 mg/kg / 10 mg/kg) and placed in a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Mice were unilaterally microinfused with either the D2R or Null vector into the NAc according to stereotaxic coordinates in the mouse brain atlas of Paxinos and Franklin (Paxinos and Franklin, 2001). The side of infusion into each mouse was selected randomly such that half received infusions into right NAc and half into left NAc.

Previous reports describe details of the vector construction (Ikari et al., 1995; Umegaki et al., 1997). Briefly, the recombinant Ad vectors were derived from Ad type 5 in which the majority of the E1 and a portion of the E3 regions had been deleted, and an expression cassette containing the rat D2R cDNA along with the cytomegalovirus (CMV) immediate early promotor and enhancer was inserted at the site of the E1 deletion. Two vectors were utilized: (1) D2R vector [(AdCMV.D2R) (10^{10} pfu/ml)], containing the rat D2R cDNA and (2) Null vector (AdCMV.Null), a similar construct, missing the D2R cDNA, as a control.

Microinfusion was carried out using an automated syringe pump (Razel, Stamford, CT) and a 26-gauge 1 ìl Hamilton microsyringe connected to a 28-gauge internal cannula. Each microinfusion administered 1 ìl of vector over 10 minutes so as to reduce the risk of procedure-induced lesions.

Behavior analysis: two bottle choice ethanol paradigm

Ethanol self-administration was monitored using the two-bottle preference test, which is a model that replicates aspects of voluntary alcohol consumption in humans (McBride and Li, 1998). Animals were individually housed and had continuous access to both ethanol and distilled water from two 10 ml graduated glass drinking bottles. Mice were acclimated to the taste and effects of ethanol through progressively increasing the concentration of ethanol (0, 3, 6, and 10% v/v). After 2 weeks of habituation to the presence of 10% ethanol, baseline ethanol drinking behavior was recorded daily (1500h) for 2 weeks. Each day the position of the ethanol bottle was reversed to control for side preferences. During this time each animal's body weight was recorded. The amount of ethanol consumed divided by total fluid consumed x 100 was used as a measure of ethanol percent preference. In addition, daily ethanol consumption was measured in g/kg/day. After 2 weeks of baseline data, animals were treated with a microinfusion of either the D2R or the Null vector into the NAc, and then returned to the two-bottle choice ethanol procedure for 18 days.

Results

D2R vector

Ethanol intake

Baseline assessment of ethanol consumption in *Drd2*$^{+/+}$ mice revealed a relatively high level of ethanol consumption, with a baseline intake average of 5.30 ± 0.52 g/kg/day (Fig. 1A). Treatment of *Drd2*$^{+/+}$ mice with D2R DNA transfer (D2R vector) resulted in a significant decrease (Fig. 1A) in ethanol drinking behavior (1-way repeated measures ANOVA; $F = 7.23$; $p < 0.001$). Subsequent

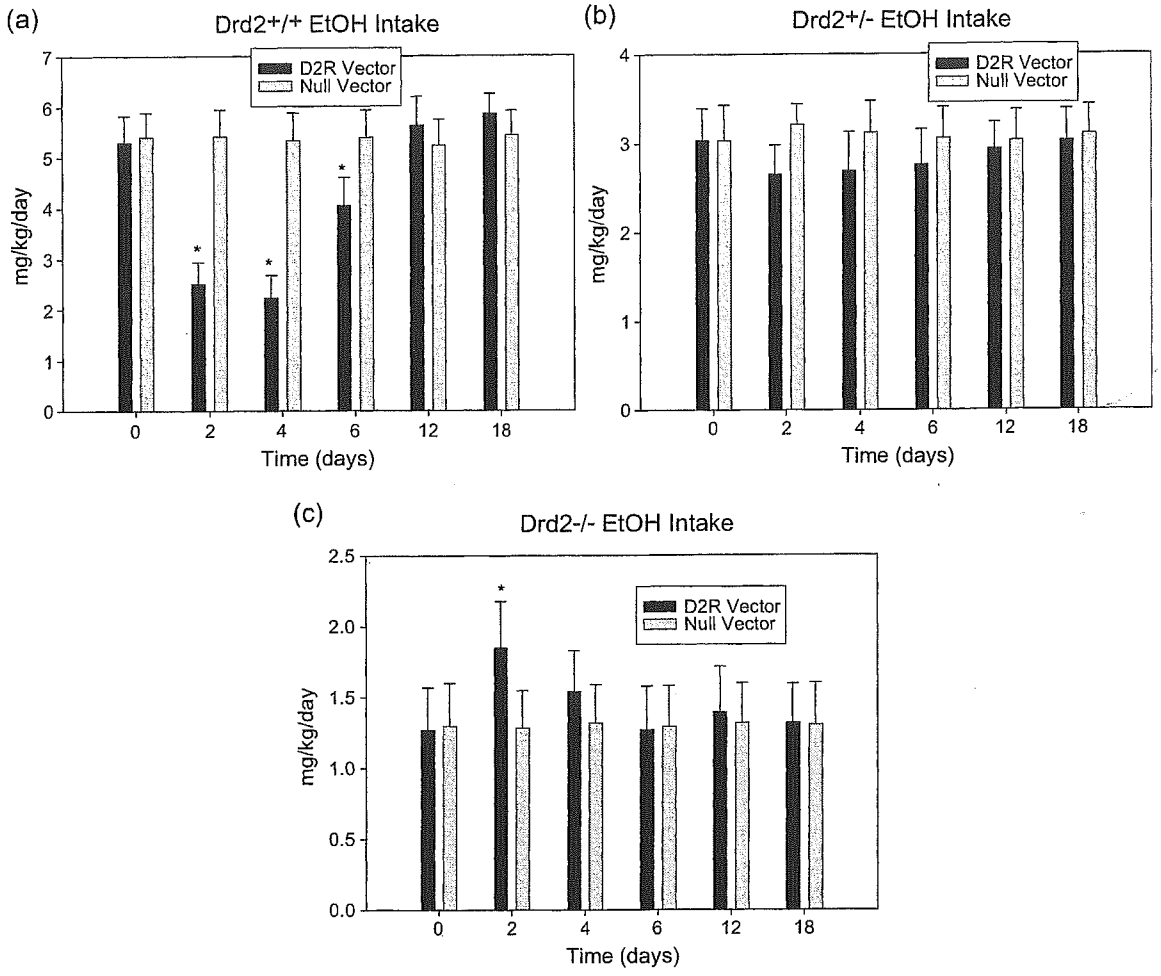

Fig. 1. Mean (+ SEM) ethanol intake (g/kg/day) over time (* $p < 0.05$) in: a) *Drd2+/+*; b) *Drd2+/–* and c) *Drd2–/–*.

analyses using paired t-tests revealed significant differences in ethanol intake between baseline (day 0) and post-D2R vector treatment, day 2 ($t = 4.67$; $p < 0.001$), day 4 ($t = 4.42$; $p < 0.002$) and day 6 ($t = 1.25$; $p < 0.04$). No significant differences were observed at 12 ($t = 0.19$; $p > 0.05$) or18 days ($t = 0.06$; $p > 0.05$).

Drd2$^{+/-}$ mice displayed a baseline ethanol intake average of 3.04 ± 0.36 g/kg/day (Fig. 1B). In these mice D2R DNA transfer did not produce a statistically significant change in ethanol consumption (1-way repeated measures ANOVA; $F = 0.20$; $p > 0.05$). Similarly, paired t-tests between baseline (day 0) ethanol intake and all post-D2R vector treatment days, did not reveal any statistically ($p > 0.05$) significant differences (day 2, $t = 1.13$; day 4, $t = 0.69$; day 6, $t = 0.57$; day 12, $t = 0.20$; and day 18, $t = 0.01$).

Drd2$^{-/-}$mice revealed a baseline mean ethanol consumption of 1.27 ± 0.30 g/kg/day (Fig. 1C). Paired t-test comparisons between baseline (day 0) and post-D2R vector treatment days, showed an initial significant increase in ethanol self-administration at day 2 ($t = 2.76$; $p < 0.02$). In contrast, paired t-

tests revealed no statistical difference ($p > 0.05$) between baseline and day 4 ($t = 0.27$), day 6 ($t = 0.01$), day 12 ($t = 0.36$) and day 18 ($t = 0.15$).

Ethanol preference

Percent ethanol preference was similarly assessed in all groups before and after treatment with D2R DNA transfer. In particular, *Drd2+/+* mice demonstrated a decline in mean % ethanol preference after treatment with the D2R vector (Fig. 2A). *Drd2+/+* mice exhibited a 64.84 ± 1.25 % ethanol preference baseline average (day 0) and when compared to post D2R vector treatment a significant difference was observed (1-way repeated measure ANOVA; $F = 11.08$; $p < 0.001$). More specifically, multiple paired t-test comparisons revealed the following differences in % ethanol preference between baseline and day 2 ($t = 2.739$, $p < 0.02$); day 4 ($t = 4.46$, $p < 0.002$); day 6 ($t = 5.38$, $p < 0.001$); day 12 ($t = 0.518$, $p > 0.05$) and day 18 ($t = 0.404$, $p > 0.05$).

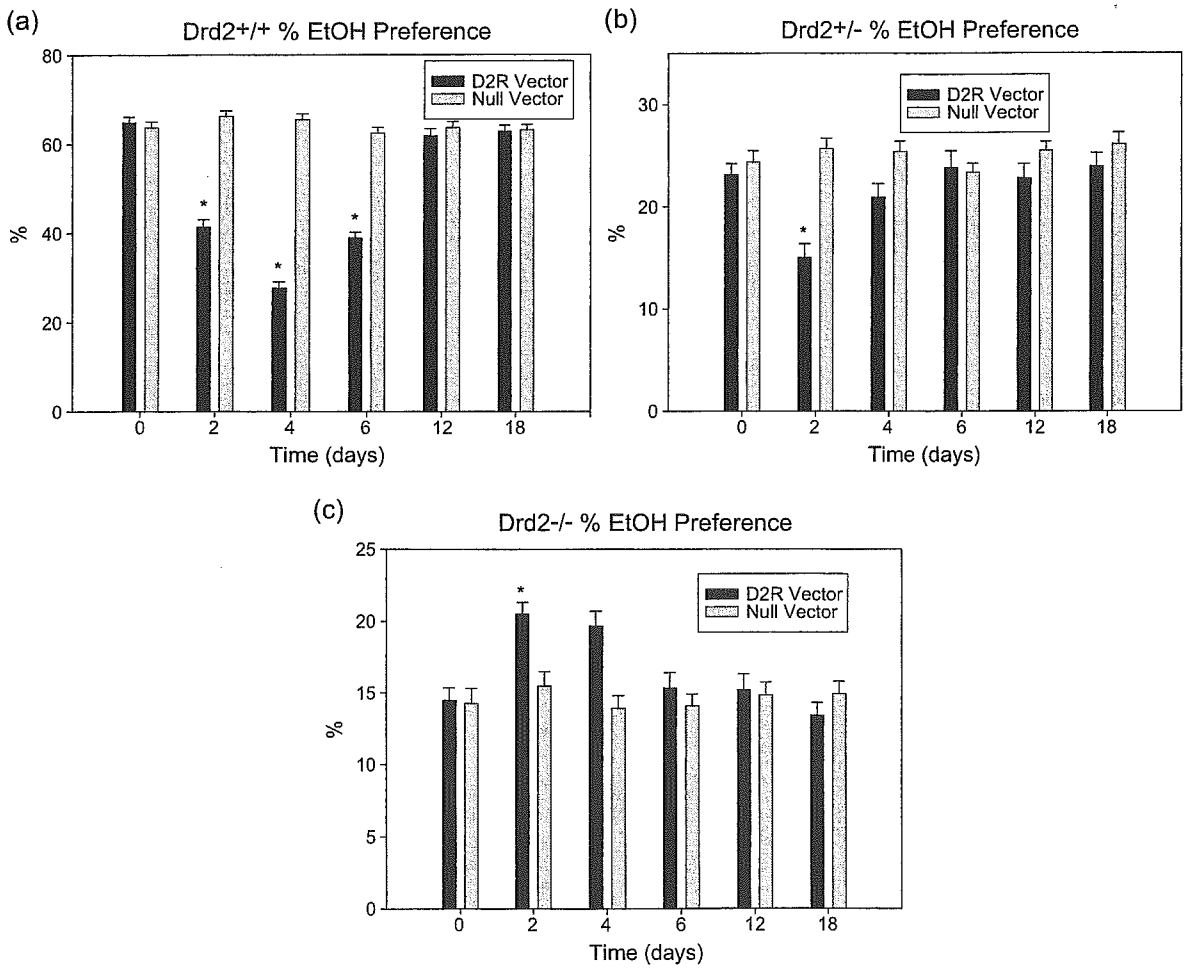

Fig. 2. Mean (+ SEM) percent ethanol preference over time (* $p < 0.05$) in: a) *Drd2+/+* ; b) *Drd2+/–* and c) *Drd2–/–*.

Drd2+/– ethanol % preference (23.13 ± 1.08) was not significantly influenced after D2R DNA transfer compared to baseline (1-way repeated measure ANOVA; $F = 1.17$; $p > 0.05$; Fig. 2B). However, subsequent multiple paired t-tests revealed the following differences in % ethanol preference between baseline and day 2 ($t = 2.700$, $p < 0.024$); day 4 ($t = 0.522$, $p > 0.05$); day 6 ($t = 0.104$, $p > 0.05$); day 12 ($t = 0.070$, $p > 0.05$) and day 18 ($t = 0.364$, $p > 0.05$).

Similarly, a 1-way repeated-measures ANOVA of the *Drd2*–/– ethanol % preference (14.48 ± 0.84) did not reveal a significant main effect of D2R DNA transfer ($F = 1.887$; $p > 0.05$; Fig. 2C). However, paired t-test comparisons revealed the following differences between baseline and day 2 ($t = 2.261$, $p < 0.05$); day 4 ($t = 1.325$, $p > 0.05$); day 6 ($t = 0.311$, $p > 0.05$); day 12 ($t = 0.228$, $p > 0.05$) and day 18 ($t = 0.698$, $p > 0.05$).

Null vector

Quantitative analyses of ethanol intake and percent preference data in the Null vector treated mice did not reveal any significant changes (Figs. 1, 2). Using a 1-way ANOVA, ethanol intake post treatment with the Null vector did not significantly differ from baseline in the *Drd2*+/+ ($F = 0.008$; $p > 0.05$), *Drd2*+/– ($F = 0.044$; $p > 0.05$) and *Drd2*–/– ($F = 0.003$; $p > 0.05$) mice. Similarly, a 1-way ANOVA was used to examine ethanol percent preference levels. Ethanol preference remained at similar to baseline levels, and did not differ statistically in the *Drd2*+/+ ($F = 0.091$; $p > 0.05$), *Drd2*+/– ($F = 0.099$; $p > 0.05$) and *Drd2*–/– ($F = 0.049$; $p > 0.05$) mice.

Discussion

Previous studies demonstrated that D2R deficient mice displayed altered levels of ethanol preference (Phillips et al., 1998), operant ethanol self-administration (Risinger et al., 2000), and ethanol conditioned place preference (Cunningham et al., 2000). In this study, we examined the effects of manipulating the levels of D2R gene expression in the NAc of *Drd2*$^{-/-}$ mice.

We observed that *Drd2*–/– mice displayed a lower baseline with respect to ethanol drinking compared to *Drd2*+/+, in agreement with previous studies(Phillips et al., 1998). Ethanol preference was 15% for *Drd2*–/– compared to 23% for *Drd2*+/– and 65% for *Drd2*+/+, and ethanol intake was 1.3 g/kg/day for *Drd2*–/– mice compared to 3 g/kg/day for *Drd2*+/– and 5.3 g/kg/day for *Drd2*+/+ mice.

Drd2+/+ mice treated with the D2R vector in the NAc displayed significant reductions in both ethanol preference (57%) and intake (58%), which was similar to the effects that the D2R vector treatment had on ethanol intake and preference in rats (Thanos et al., 2001, 2004). Maximum attenuation of both preference and intake was observed 4 days after treatment with the D2R vector and lasted approximately one week. D2R DNA transfer in *Drd2*+/– mice revealed an intermediate level of attenuation (35%) in ethanol preference. In contrast, D2R DNA transfer in the *Drd2*–/– mice increased ethanol intake (46%), and preference (42%). This effect was, however, short lasting with baseline drinking resuming by day 4 post treatment. We interpret the influence of genotype on the drinking behavior of C57Bl/6J following the manipulation of D2R number to imply that the presence of D2R is necessary for the expression of ethanol consumption, and that some threshold level of D2R expression in the NAc is required for the reinforcing effects of ethanol to occur. The differences in the baseline levels of alcohol consumption between the *Drd2*–/–, *Drd2*+/– and *Drd2*+/+ mice and the

decreases in alcohol consumption with D2R over expression suggested an inverted u shape curve between the levels of D2R and alcohol consumption in these mice.

While examination of the role of D2R on conditioned association and learning was beyond the scope of the present study, it has been previously demonstrated that D2R in the NAc participates in the neuronal coding for specific types of responses to incentive contingencies and in spatial learning (Tran et al., 2002). More specifically, one of the two isoforms of the D2 receptor, termed D2L (long form) has been suggested to be critical in the acquisition (learning) and/or retention (memory) of context-stimulus associations (Smith et al., 2002) critical in understanding the neurobiological basis of alcoholism.

Future studies examining the effect(s) of chronic ethanol consumption on *Drd2+/+* , *Drd2+/−* and *Drd2−/−* mice will seek to assess whether the three genotypes differ with respect to their ability to learn to self administer alcohol, regardless of intake levels. Secondly, how if at all, is drinking behavior influenced long term (beyond 4 weeks post treatment); that is, if examined for a longer period of time, would *Drd2+/−* mice display an escalation in alcohol consumption? If our hypothesis remains intact D2R should be required for a conditioned association between drinking alcohol and its reinforcing effects to be formed, and that low D2R levels will inhibit learning this association, as well; total lack of D2R should produce even more pronounced deficit with respect to association learning. In addition, we hypothesize that once association learning has been established, reduced D2R levels should render the animal more vulnerable to more elevated levels of ethanol drinking. In the present study, *Drd2+/−* mice drank less than the *Drd2+/+* mice; which does not support our hypothesis; it is possible that conditions in the current study were not optimal to observe compulsive administration.

In summary, these results provide further insight into the complex role that D2R levels play in alcohol intake. Furthermore, these data help us better understand previous findings using D2R mice and differences in alcohol consumption, and support the notion of a critical threshold (Bmax) of D2R. It is possible that significant deviation from this optimum level, could be reflected behaviorally either as an attenuation or potentiation of alcohol drinking. In addition, these results may help us better understand the mechanism(s) of alcohol abuse and may well assist in the development of specific molecular based treatments of alcoholism. While the use of vector delivered sequences in alcohol research (as performed in this study) are very promising, several limitations remain (Israel et al., 2002). Future clinical studies involving the treatment of alcoholism using DNA transfer may provide an opportunity for utilizing less invasive, more specific, and safer approaches (through the use of nonviral or gutless vectors) (Israel et al., 2002). Also, as we learn more about D2Rs, and their involvement, in the mechanism of alcohol preference and addiction we will begin to better understand the relationship between the actual amount of D2R levels and ethanol drinking behavior, it may be possible to develop more effective interventions.

Acknowledgments

The authors thank, Katherine Suchland, Andrew Lee, Brittney Tejada, Maryann Kershaw and Dr. Bruce Scharf for assistance with this study and for veterinary care. We would also like to thank Brian Murfin, Kevin Manning and the SULI program at Brookhaven National Laboratory for supporting KW. This work was supported by the NIAAA (AA 11034 & AA07574, AA07611), NIMH (MH66360 & MH67497) and NIDA (DA12062) and by the U.S. Department of Energy under contract DE-AC02-98CH10886.

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Psychopharmacology (2005) 182: 253–261
DOI 10.1007/s00213-005-0072-x

ORIGINAL INVESTIGATION

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Dopamine D_2 receptor plays a role in memory function: implications of dopamine–acetylcholine interaction in the ventral hippocampus

Received: 12 February 2005 / Accepted: 9 May 2005 / Published online: 16 July 2005
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Abstract *Rationale:* The role of the hippocampal dopaminergic system in mnemonic function has not been clarified yet. *Objective:* We previously reported that the dopamine D_2 receptor (D_2R) is involved in the regulation of acethylcholin (ACh) release in the hippocampus. In this study, we further investigated ACh–dopamine (DA) interaction in the hippocampus and its involvement in mnemonic function. *Methods:* For experiment 1, rats fed with Cholin (Ch)-deficient chow were used. We examined the effects of D_2R antagonist, raclopride, on cognitive performance using a passive avoidance task. We further carried out in vivo microdialysis to assess the effect of infusion of D_2R agonist, quinpirole, into the ventral hippocampus on its capacity to release ACh. For experiment 2, rats fed with normal chow were used. The performance of a radial arm maze task was assessed to examine the effects of hippocampal injection of D_2R agonist, quinpirole, on memory impairment induced by scopolamine, a muscarinic ACh antagonist. *Results:* In experiment 1, rats fed with Ch-deficient chow showed impaired performances indicated by prolonged latency on retention trials of a passive avoidance task following the hippocampal injection of D_2R antagonist, and showed reduced capacity to release ACh following the injection of D_2R agonist compared with rats fed with normal chow. In experiment 2, memory impairment induced by the intraperitoneal injection of scopolamine was ameliorated by the injection of D_2R agonist into the ventral hippocampus. *Conclusion:* These results indicate the possible involvement of hippocampal ACh–DA interaction in mnemonic processing.

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Keywords Radial arm maze · Passive avoidance · In vivo microdialysis · Quinpirole · Raclopride · Scopolamine

Abbreviations D_2R: dopamine D2 receptor · ACh: acetylcholine · DA: dopamine · Ch: Choline · AChE: Acetylcholine esterase · AChEIs: Acetylcholine esterase inhibitors · ACSF: Artificial cerebrospinal fluid · HPLC: High-performance liquid chromatography · HPLC–ECD: high-performance liquid chromatography system with electrochemical detection · PBS: phosphate buffer saline · ANOVA: analysis of variance · DH: dorsal hippocampus · VH: ventral hippocampus

Introduction

It is well known that cholinergic basal forebrain neurons projecting to the cerebral cortex and hippocampus play a crucial role in learning and memory (Mufson et al. 2003; Paul 2003), and a number of studies have shown that disturbances in the cholinergic system induce learning and memory impairment (Day et al. 1991; Wilson and Cook 1994; Walsh et al. 1996; Mishima et al. 2001; Rogers and Kesner 2003). We previously demonstrated that the dietary restriction of Choline (Ch), which is a precursor of acetylcholine (ACh), induces reduced capacity to release ACh in the hippocampus, as confirmed by in vivo microdialysis, and impairs performance of a passive avoidance task in rats (Nakamura et al. 2001).

On the other hand, attention has been paid to the involvement of hippocampal dopamine (DA) systems, especially the dopamine D_2 receptor (D_2R), in mnemonic function. Hippocampal DA neurons project from the ventral tegmental area, with some DA fibers in the posterior hippocampus originating from the substantia nigra (Verney et al. 1985). In fact, several studies have shown that disturbances in dopaminergic systems induce learning and memory impairment in rats. For example, Gasbarri et al. (1996) revealed that direct hippocampal injection of 6-hydroxydopamine causes selective lesions of the mesencephalic dopaminergic system, which in turn induce learning

and memory impairment. Using a radial arm maze, Wilkerson and Levine (1999) demonstrated that D_2R in the ventral hippocampus is involved in spatial memory. In addition, an electrophysiological study showed that a D_2R antagonist leads to disturbances of long-term potentiation, a key phenomenon involved in memory consolidation, in the rat hippocampus (Frey et al. 1990; Yanagihashi et al. 1991).

The ACh–DA interaction in the striatum has been thoroughly investigated (Acquas and Chiara 2002; Kurotani et al. 2003). However, only a few studies have addressed the ACh–DA interactions in the hippocampus. Although it has been well documented that ACh–DA interactions play a role in mnemonic processing by studies with systemic drug injection (Levin et al. 1989; McGurk et al. 1989), to date, there has not been a study using focal injections to investigate the direct involvement of the hippocampal D_2R in this interaction. We previously demonstrated that a D_2R agonist dose-dependently stimulates acetylcholine (ACh) release in the ventral hippocampus; in that study, in vivo microdialysis and the results of a 14-unit T-maze (Stone maze) task suggested that D_2R in the ventral hippocampus was involved in mnemonic function via ACh release (Umegaki et al. 2001).

In the present study, we carried out a series of experiments to further investigate ACh–DA interactions in the hippocampus. In our first experiment using a passive avoidance task, rats were fed with Ch-deficient chow, which was confirmed to show a reduced capacity in releasing ACh in the hippocampus (Nakamura et al. 2001), and the effects of hippocampal injection of a dopamine D_2R antagonist on cognitive performance were observed. We also carried out in vivo microdialysis in rats fed with Ch-deficient chow, and examined the effects of the dietary manipulation of Ch on the capacity to release ACh from the ventral hippocampus induced by the infusion of a D_2R agonist into the ventral hippocampus (experiment 1). Then, by assessing the performance of rats fed with normal chows on a radial arm maze task, we examined whether memory impairment induced by scopolamine, a muscarinic ACh antagonist, could be alleviated by the intrahippocampal administration of a D_2R agonist (experiment 2).

Materials and methods

Experiment 1

Subjects

Male 9-week-old Wistar rats, housed in cages maintained at an appropriate temperature (20°C) and a 12-h light/dark cycle (7 A.M./7 P.M.), were used. The animals were assigned at random to one of two dietary groups. Rats assigned to the control group were fed a diet of chow containing 0.2% Ch. The remaining rats were fed a diet deficient in Ch that contained less than 0.03% (i.e., below the level of detection). Each diet was administered to the animals for 12 weeks. Both diets had the same composition of ingredients, with the exception of the Ch content, as follows: (casein 6.0%, starch 71.5%, sugar 10.0%, soybean oil 0.5%, fatty oil 4.0%, cellulose 3.0%, United States Pharmacopoeia-vitamin: 1.0%, Funabashi Farm, Chiba, Japan). The rats were allowed free access to food and water ad libitum, and they were housed in a temperature-controlled (20°C) facility.

All experiments were conducted according to the protocols approved by the Animal Care and Use Committee of Nagoya University. All efforts were made to minimize the number of animals used and their suffering.

Cannula implantation

The rats were anesthetized with pentobarbital (45 mg/kg) and fixed to a stereotaxic frame. The skull was exposed, and guide cannulae (31-90100, BAS, Tokyo, Japan) were bilaterally placed into the ventral hippocampus at the following coordinates: AP −5.0 mm, ML±4.8 mm, DV 7.0 mm, with reference to bregma and the dural surface in accordance with the atlas of Paxinos and Watson (1986). A recovery period of 3 days was allowed before the test. All rats underwent the cannulae implantation, and were then divided into two groups. The first group (n=42) was subjected to a behavioral study, and the second group (n=12) was subjected to an in vivo microdialysis study.

Histology

After completion of the experiment, the animals were injected 1 μl of cresyl violet dye to confirm cannula placement. The animals were anesthetized with ether and decapitated. After the brain was removed, it was frozen and sliced to a thickness of 40 μm. The injected sites were confirmed by macroscopic examination. Only data from animals in which the injections were made into the desired sites were analyzed.

Behavioral study

Passive avoidance task Both the Ch-deficient rats and controls were habituated to a two-compartment shuttle box on 2 successive days before being subjected to a step-through passive avoidance task. The apparatus used for the task was composed of two compartments of equal size (25×25×25 cm), separated by a common wall. One of the two compartments was brightly illuminated from above by two 12-W bulbs. The other compartment was not illuminated and had an electrifiable grid floor. A guillotine-type door (15×10 cm) was located in the center of the common wall in order to allow rats to move about freely between the compartments (Nakamura et al. 2001). When habituation was completed, the rats were placed in the dark compartment and were then tested for the acquisition of passive avoidance. In brief, the rat was first placed in the light compartment. When the rat entered the dark com-

partment, the door was closed, and an electric shock to the foot (AC 1 mA) was immediately applied via the floor grids for 3 s. The rat remained in the dark chamber for 30 s after the foot shock had been administered, and was then removed from the apparatus and temporarily placed in a holding cage for 120 s. The rat was then returned to the light compartment and tested for the assessment of memory acquisition. The successful acquisition of passive avoidance was recorded when the rat remained in the light compartment for 300 s without entering the dark compartment. If this criterion was not met, upon entering the dark compartment, the rat received the same foot shock. The number of trials required for meeting the criterion was recorded for comparison between the four groups. Retention of passive avoidance was tested 72 h after the acquisition trial. The time until the rats moved to the dark compartment was recorded as latency, for up to a maximum of 300 s (Eidi et al. 2003).

Drug administration A 27-gauge stainless steel injection cannula was used for infusion of the drug. The cannula was connected to a CMA/100 microinjection pump (BSA, Tokyo, Japan) via polyethylene tubing. Either phosphate buffer saline (PBS) or raclopride dissolved in PBS (500 μg/ml in concentration) was infused into the bilateral ventral hippocampi at an injection speed of less than 1.0 μl/min for 10 min in order to avoid tissue destruction. The total volume used for the infusion depended on the body weight of rats (80 μl/kg). The infusion was implemented 20 min prior to both the acquisition and retention of passive avoidance trials. Four different groups were thus created according to diet content and agents administered into the ventral hippocampus. The following groups were used: control diet–PBS (control+PBS), control diet–raclopride (control+raclo), Ch-deficient diet–PBS (Ch-d+PBS) and Ch-deficient diet–raclopride (Ch-d+raclo). After eliminating data from two rats with missed cannula placement and one rat with brain bleeding, the final four group sizes were: control+PBS, n=10 (329.6± 21.3 g); control+raclo, n=11 (337.4±18.5 g); Ch-d+PBS, n=10 (339.3±17.2 g); Ch-d+raclo, n=8 (322.7± 34.6 g). There was no statistically significant difference in body weight among the four groups during the experiments. The injection cannulae were left in place for 2 min following administration to allow the drug to diffuse away from the tip.

In vivo microdialysis study

Microdialysis procedure A perfusion procedure was carried out 72 h after the operation in order to avoid the effects of anesthesia and surgery. A microdialysis probe (31-9503; BAS) was inserted through the guide cannula and a perfusion pump was connected to the probe, according to a procedure devised to allow the rats to move freely in a hemispheric Plexiglass box. We used artificial cerebrospinal fluid (ACSF), which had the following composition: glucose 4 mmol/l, NaCl 126 mmol/l, KCl 4.0 mmol/l, KH_2PO_4 1.4 mmol/l, $MgSO_4$ 1.3 mmol/l, $CaCl_22H_2O$ 2.4 mmol/l, $NaHCO_3$ 26 mmol/l. Physostigmine (eserine hemisulfate; 100 μmol/l) (Sigma, St. Louis, MO, USA) was added to the solution, and was refluxed at a flow rate of 2 μl/min. The collected perfusates were sampled every 20 min. At 80 min after the initiation of the perfusion, the perfusate was exchanged for ACSF containing quinpirole (100 μmol), a D_2R agonist. The perfusion was continued for another 80 min. The collected samples, mixed with 1 pmol of ethylhomocholine (BAS) as an internal standard, were immediately subjected to measurement of ACh levels using an HPLC assay system (BAS). After eliminating data from one rat with missed cannula placement, the final group sizes for the two dietary manipulation studies were: control diet group (n=6, 334.3± 19.8 g) and Ch-deficient diet group (n=5, 317.0±9.0 g). There was no statistically significant difference in body weight between two diet groups during the experiment.

High-performance liquid chromatographic assay Twenty microliters of sample perfusate was injected into a high-performance liquid chromatographic system with electrochemical detection (HPLC–ECD) without further purification (BAS). Two separate HPLC–ECD systems were used. Each contained a pump (PM-60), a degasser (CD-22p), a heating box (FT-1), and an electrochemical detector (LC-4C). Acetylcholinesterase and choline oxidase were immobilized on an enzyme cartridge column (BAS). The potential of the platinum electrode was kept at +450 mV vs a Ag/AgCl reference electrode. The mobile phase consisted of 50 mM $Na_2HPO_2{\cdot}12H_2O$ containing 1 mM EDTA 2Na. The pumping rate of the mobile phase was 80 μl/min.

Statistical analysis

The number of trials required for the acquisition of passive avoidance and the latency in retention trials were compared using the Kruskal–Wallis test followed by the Mann–Whitney U-test. The data obtained from the HPLC assay for the determination of ACh levels were expressed as a percentage of the baseline concentrations in the perfusates. Differences in ACh output between groups (controls and Ch-deficient groups) were statistically analyzed using a repeated-measures analysis of variance (ANOVA).

Experiment 2

Subjects

Male 9-week-old Wistar rats, weighing 250–300 g and housed in a cage at an appropriate temperature (20°C) and a 12-h light/dark cycle (7 A.M./7 P.M.), were used. Body weight was maintained at approximately 80% of the initial weight, with dietary restrictions imposed during the experimental period.

All experiments were conducted according to protocols approved by the Animal Care and Use Committee of Nagoya University. All efforts were made to minimize the number of animals used and their suffering.

Apparatus

We used a modified eight-arm radial maze originally developed by Olton and Samuelson (1976) for behavioral testing. The maze was placed 50 cm above floor level. It consisted of a central platform, 40 cm in diameter, with eight arms (12 cm in width, 80 cm in length) extending radially. Each arm was surrounded by opaque plastic side walls (5 cm in height). Guillotine-type doors were located between the central platform and each extending arm. Food cups that had food pellets in the middle, used as reinforcers, were placed near the end of each arm. The experimental room contained extra-maze visual cues that surrounded the maze.

Preparation of animals for the eight-arm radial maze

Before the trial, all rats were allowed to freely explore the maze in order to enable them to become habituated to the apparatus. Habituation was carried out three times a day (10 min each) for 1 day prior to the training session. One day following habituation, training sessions were carried out three times a day, up to a maximum of 4 days. In each training session, the animal was placed on a platform in the middle of the eight radial arms. After 10 s, the doors were lifted, and the animal was allowed to move freely about the maze. Each time the rat returned to the platform, the doors were closed for 10 s and then lifted again. The performance of a given animal on each trial was assessed on the basis of three parameters: the number of correct choices among the eight arms initially chosen, the number of errors (defined as the choice of arms that had already been visited), and the time elapsed before the animal ate all eight pellets. If the animals made seven or eight correct choices and less than one error in three successive sessions within 5 min, they were subjected to no further trials.

Surgery

In the training sessions, if the rats meet the above criteria, they were subjected to the operation for cannulation. They were anesthetized with pentobarbital (45 mg/kg) and were fixed to a stereotaxic frame. The skull was exposed, and guide cannulae (31-90100; BAS) were placed into the bilateral ventral hippocampus at the following coordinates: AP −5.0 mm, ML±4.8 mm, DV 7.0 mm, with reference to the bregma and the dural surface in accordance with the atlas of Paxinos and Watson (1986). A recovery period of 3 days was allowed. Before the drug administration trials, the rats underwent the training procedure to confirm that they again met the above criteria following surgery for a maximum of 2 days.

Histology

After completion of the experiment, the animals received injections of 1 μl cresyl violet dye to confirm cannula placement. The animals were anesthetized with ether and decapitated. After the brain was removed, it was frozen and sliced to a thickness of 40 μm. The injected sites were confirmed by macroscopic examination. Only data from animals in which the injections were made into the desired sites were analyzed.

Drug administration

A 27-gauge stainless steel injection cannula was used for infusion of the drug. The cannula was connected to a CMA/100 microinjection pump (BSA, Tokyo, Japan) via polyethylene tubing. Drugs were administered once in a single trial for each rat. At 30 min before the trial, either scopolamine (0.125 mg/kg) or PBS was administered intraperitoneally. Rats that were administered PBS served as controls. As regards the rats that were given scopolamine, either quinpirole (8 or 16 μg/kg), quinpirole+raclopride (simultaneously 16 μg/kg each), or PBS was injected into the ventral hippocampus bilaterally 20 min prior to the trial. Thus, five different groups were created, depending on the agents administered both intraperitoneally and intracerebrally: PBS–PBS (controls), scopolamine–PBS, scopolamine–quinpirole (8 μg/kg), scopolamine–quinpirole (16 μg/kg), and scopolamine–quinpirole+raclopride (16 μg/kg each). The total injection volume depended on body weight (16 μl/kg each) and the rate of injection was less than 1.0 μl/min over a 10-min period avoiding mass effects. The injection cannula was left in place for 2 min in order to allow the drug to diffuse away from the tip. After eliminating data from two rats with missed cannula placement, the final five group sizes were: controls (n=7, 246.8±15.7 g); scopolamine–PBS (n=7, 242.1±19.3 g); scopolamine–quinpirole (8 μg/kg) (n=8, 246.8±21.3 g); scopolamine–quinpirole (16 μg/kg) (n=8, 231.3±15.7 g); scopolamine–quinpirole+raclopride (16 μg/kg each) (n=5, 228.0±15.2 g). There was no statistically significant difference in body weight among the five groups during the experiment.

Statistical methods

The number of correct choices in the eight arms initially chosen (i.e., the initial response number) was compared using the Kruskal–Wallis test followed by Mann–Whitney U-test to examine significant differences. The average time spent by each rat visiting each arm among four groups statistically was compared using one-way ANOVA.

Results

Experiment 1

Behavioral studies

There was no significant difference noted between groups with respect to the number of trials required for the acquisition of passive avoidance behavior (Fig. 1). Regarding the retention of nociceptive memory, which was assessed by the latency period until the animal stepped into the dark compartment of the shuttle box, Fig. 2 shows the effect of PBS or raclopride on the respective diet groups. The Ch-deficient diet–raclopride (Ch-d+raclo) group had a shorter latency on retention trials than the other three groups ($P<0.01$, Kruskal–Wallis test followed by Mann–Whitney U-test). There was no significant difference among the three groups (control+PBS, control+raclo, and Ch-d+PBS). Therefore, neither dietary manipulation of Ch intake nor focal injection of raclopride alone affected the latency time in this retention trial.

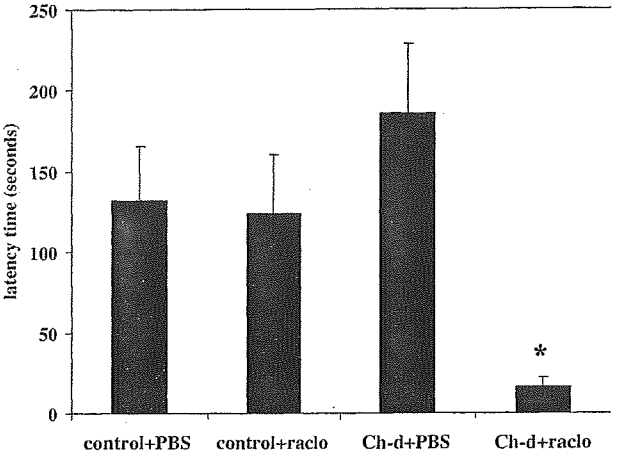

Fig. 2 The latency time (mean±SEM) in retention by the effect of PBS or raclopride on the respective diet groups. Only Ch-deficient diet–raclopride (Ch-d+raclo) group had shorter latency on retention trials than the other three groups (*$P<0.01$, Kruskal–Wallis test followed by Mann–Whitney U test). There was no significant difference among the following three groups: control+PBS, control+raclo, and Ch-d+PBS (control+PBS, n=11; control+raclo, n=10; Ch-d+PBS, n=10; Ch-d+raclo, n=8)

Microdialysis studies

As shown in Fig. 3, the increase in ACh release in the ventral hippocampus induced by quinpirole was reduced in the Ch-deficient diet group compared with the control group ($P<0.05$, repeated-measures ANOVA).

Experiment 2

As shown in Fig. 4, the number of correct choices from among the eight arms initially visited differed significantly in the scopolamine–PBS injected group and the scopolamine–quinpirole+raclopride (16 μg/kg each) injected group compared with controls (PBS–PBS injected) ($P<0.01$, Mann–Whitney U-test). Both doses (8 and 16 μg/kg) of scopolamine–quinpirole significantly increased the number of correct choices compared with the scopolamine–PBS injected group and the scopolamine–quinpirole+raclopride (16 μg/kg each) injected group (each $P< 0.05$, $P<0.01$, Mann–Whitney U-test). On the other hand, there was no significant difference between the scopolamine–PBS group and the scopolamine–quinpirole+raclo-pride group (16 μg/kg each). A focal injection was administered into the ventral hippocampus in order to avoid the other DA-related effects (e.g., motivation or locomotion). No statistically significant difference was observed between the four groups that received scopolamine (i.p.) with respect to their locomotor

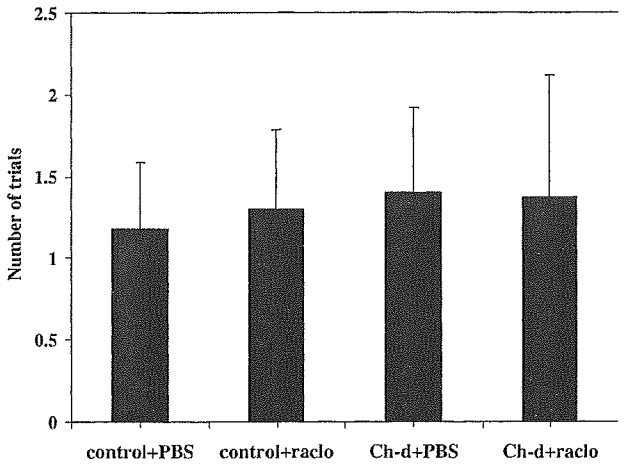

Fig. 1 The number of trials (mean±SEM) required for the acquisition of passive avoidance behavior. There was no statistically significant difference between the four groups (control+PBS, n=11; control+raclo, n=10; Ch-d+PBS, n=10; Ch-d+raclo, n=8)

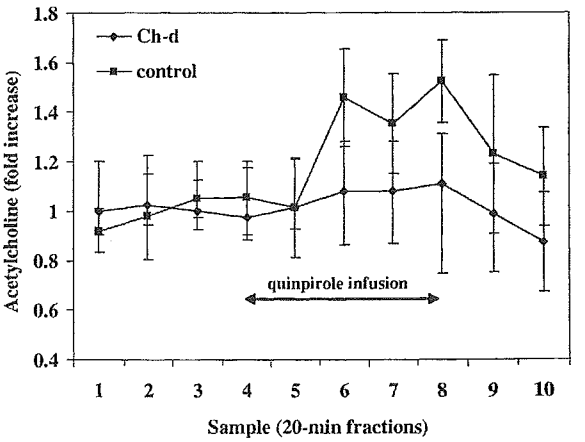

Fig. 3 Acetylcholine release induced by quinpirole injection in the ventral hippocampus. The *data points* represent group means±SEM. A significant difference was observed between Ch-deficient and control rats (control, n=6; Ch-d, n=5; $P<0.05$, a repeated measures ANOVA)

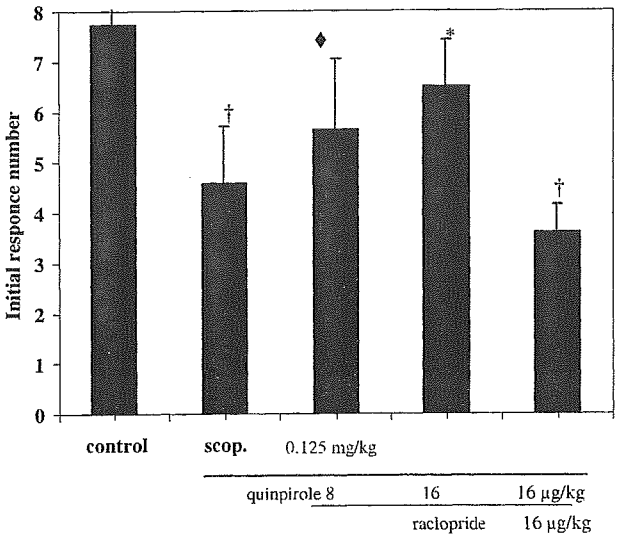

Fig. 4 Effects of intrahippocampal injection of quinpirole and raclopride on scopolamine-induced impairment of memory in the eight-arm radial maze. Scopolamine (scop.; 0.125 mg/kg) and quinpirole (+raclopride) were injected 30 min, intraperitoneally, and 20 min, intracerebrally, prior to the test, respectively (†$P<0.01$ vs control; *$P<0.01$, ◦$P<0.05$ vs scop.+PBS, scop.+quinpirole 16 μg +raclopride 16 μg; Kruskal–Wallis test followed by Mann–Whitney U test). PBS+PBS (control), n=7; scop.+PBS, n=7; scop.+quinpirole 8 μg, n=8; scop+quinpirole 16 μg, n=8; scop.+quinpirole 16 μg +raclopride 16 μg, n=5

speed as they visited each arm of the maze (Table 1). There was no significant difference between the group administered with the intrahippocampal PBS injection and the other groups, according to Scheffe's post-hoc test.

Discussion

Using a 14-unit T maze (Stone maze), we previously demonstrated that maze learning was impaired in rats following ventral hippocampal injection of raclopride, a D_2R antagonist; this impairment was reversed when quinpirole, a D_2R agonist, was administered simultaneously (Umegaki et al. 2001). These findings suggest the possible involvement of hippocampal D_2R dopaminergic systems in mnemonic function. This behavioral study showed the detrimental effects of a hippocampal injection of raclopride on mnemonic performance only when the rats were subjected to a chronic deficiency of dietary Ch, i.e., when the ability to release ACh upon increased neuronal demand was presumably reduced, as confirmed in our previous report (Nakamura et al. 2001). Neither dietary manipulation of Ch intake nor focal injection of raclopride alone affected behavioral performance, while Nakamura et al. (2001) showed that rats fed with Ch-deficient diet had deteriorated retention ability. This inconsistent result is attributable to a difference in behavioral assessments. They assessed the number of days it took for the rats to move into the dark compartment within maximum latency, but not latency time 72 h after the acquisition trial. Assessment of the duration of retaining nociceptive memory would probably be more sensitive to dietary manipulation. We previously demonstrated that quinpirole stimulated ACh release dose-dependently in the ventral hippocampus (Umegaki et al. 2001), and the present in vivo microdialysis study revealed that the increased ACh release in the ventral hippocampus induced by the focal administration of quinpirole was reduced under conditions of chronic dietary Ch deficiency. We believe that these findings, when taken together with the results from the current behavioral experiments, provide evidence of the involvement of ACh–DA interaction in mnemonic processing in the ventral hippocampus of rats.

As confirmed in experiment 2, quinpirole ameliorated scopolamine-induced spatial memory impairment in a dose-dependent manner, and co-injection with raclopride attenuated the ameliorative effect of quinpirole. Scopolamine has been widely known to produce acute cholinergic dysfunction, which is reflected in behavioral deficits in the performance of eight-arm radial maze observed after the intrapenitoneal administration of scopolamine (Zhang and O'Donnell 2000; Mishima et al. 2001; Daniel et al. 2003). In another study using an eight-arm radial maze task, Mishima et al. (2000) demonstrated a correlation between the behavioral changes (initial response number) induced

Table 1 The average time spent until rats entered each arm

Hippocampal injection agents	Time (seconds)	
PBS	100.60 ± 24.8	N.S.
Quinpirole (8 μg/kg)	82.85 ± 37.2	
(16 μg/kg)	73.01 ± 19.5	
Quinpirole+Raclopride (16 μg/kg each)	141.1 ± 42.2	

No significance difference using a one-way ANOVA followed by Scheffe's post hoc test

(n=5-8; mean± SEM)

No significant difference was observed among the four groups administered (i.p.) with scopolamine. No significant difference was found using one-way ANOVA followed by Scheffe's post-hoc test (n=5–8; mean± SEM)

by the systemic injection of scopolamine and changes in ventral hippocampal ACh release in rats. The present results indicate that memory impairment, once induced by acute cholinergic dysfunction, was reversed by the enhancement of dopaminergic transmission. Thus, the ameliorative effect of quinpirole on mnemonic impairment observed in the present study may have been achieved via ACh–DA interaction. The average time spent by rats on each arm in the radial arm maze task was also not affected by the intracerebral administration of dopaminergic agents (Table 1). Therefore, we concluded that the ameliorative effect of the D_2R agonist on mnemonic impairment did not improve locomotor activity, which would be expected when a dopaminergic agonist is administered systemically (Millan et al. 2004). Moreover, Gimenez-Llort et al. (2002) also showed that local raclopride administration to the ventral hippocampus induced no significant changes in locomotor activity.

A number of anatomical, physiological, and behavioral studies (Amaral and Winter 1989; Moser et al. 1993; Trivedi and Coover 2004) have indicated that the hippocampus is functionally heterogeneous along the dorsal–ventral axis; therefore, hippocampal formation is divided into two subregions of the ventral hippocampus (VH) and the dorsal hippocampus (DH). The VH contains the highest density of hippocampal dopamine innervation (Verney et al. 1985). Furthermore, D_2R was shown to be abundant in the VH (Bruinink and Bischoff 1993). Therefore, in the current study, dopaminergic agents were injected into the VH, but not into the DH, and we demonstrated that D_2R in the VH was associated with mnemonic function. On the other hand, it is generally known that the DH, but not the VH, plays an important role in learning and memory, and in particular in spatial memory (Bannerman et al. 2004). However, the relationship of the VH to mnemonic function shown in the present study could also account for recent experimental findings by other research groups. Lorenzini et al. (1996, 1997) showed that a functional inactivation of the rat VH by means of local tetrodotoxin administration induced memory impairment in a passive avoidance task. It was reported that nicotinic antagonist administration to the VH induced spatial memory impairment in rats performing tasks in a radial-arm maze (Felix and Levin 1997; Kim and Levine 1996; Levin et al. 2002). Also, Mishima et al. (2000) revealed that scopolamine administration to the VH induced spatial memory impairment in rats performing a radial-arm maze task. Such experimental evidence has indicated that the VH is associated with learning and memory, at least in the performance of passive avoidance tasks and radial-arm maze tasks. The VH is closely associated with memory-related brain sites, such as the prefrontal cortex (Bannerman et al. 2004). Moreover, the extensive connectivity between the dorsal and ventral hippocampus may also be reflected in some degree of functional interdependence between the two subregions (Amaral and Winter 1989). Further investigation of the involvement of the dopaminergic system in memory function in the VH is still necessary.

Both Alzheimer's disease and dementia with Lewy bodies, two major types of senile and degenerative dementia, are known to be associated with severe cholinergic deficits. Thus, cholinergic enhancers, represented by AChEI, remain a mainstay of pharmacological therapeutics for these diseases (McKeith et al. 1996, 2004; Cummings 2004). Meanwhile, both the pathological and clinical evidence reported to date are suggestive of the involvement of dopaminergic systems in the clinical course of dementia. For example, a loss of hippocampal D_2R in Alzheimer's disease and its correlation with memory have been reported (Joyce et al. 1993; Kemppainen et al. 2003). Empirically, anti-dopaminergic agents have been conveniently used to control various psychological and behavioral symptoms that occur with the progression of dementia (American Psychiatric Association 1997). However, the use of major tranquilizers, in particular D_2R antagonists, reportedly deteriorates cognitive function in patients with dementia (Mcshane et al. 1997). In agreement with such findings, chronic administration of haloperidol, a non-specific D_2R antagonist, reduces cholinergic fiber immunoreactivity in the rat hippocampus and impairs spatial learning performance of rats performing a water maze task (Terry et al. 2003; Parikh et al. 2004). The present findings appear to indicate that the excessive use of dopaminergic antagonists might potentially exacerbate cognitive decline in patients with dementia. On the other hand, the ameliorative effect observed in this study may suggest that dopaminergic agents could potentially be of therapeutic value in patients with cholinergic deficits, e.g., those with Alzheimer's disease, dementia with Lewy bodies.

Conclusions

The present study indicated the possible involvement of hippocampal ACh–DA interactions in mnemonic processing, as suggested by the finding that the retention of passive avoidance behavior was impaired by the focal injection of a D_2R antagonist into the ventral hippocampus; however, this finding was observed only when the cholinergic system was compromised by dietary manipulation. Furthermore, it was revealed that memory impairment induced by injection of scopolamine could be ameliorated by the focal injection of a D_2R agonist into the same site, i.e., the ventral hippocampus. Thus, the present findings may help us to better understand the therapeutic significance of dopaminergic agents in the treatment of patients with dementia associated with cholinergic deficits.

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