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Low dose domoic acid in neonatal rats abolishes nicotine induced conditioned place preference during late adolescence

Short Communication

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Summary. In this study, neonatal rats were chronically exposed to low, non-convulsive doses of the kainate receptor agonist domoic acid (DOM), or saline. Later, as adolescents, all animals were tested in a nicotine-induced conditioned place preference (CPP) paradigm. As expected, a nicotine-induced CPP was evident in the adolescent control rats, but surprisingly, not in the DOM animals. This study demonstrates the importance of KA receptors in the development of normal adolescent behaviors manifested in response to the rewarding properties of nicotine.

Keywords: Kainate receptors – Reward behaviour – Brain development – Mesocorticolimbic pathway – Addiction

Introduction

Ionotropic glutamate receptors are expressed throughout the mesocorticolimbic pathway, and have been shown to modulate dopamine release (Mathé et al., 1998; Legault and Wise, 2001; Crowder and Weiner, 2002). For instance, KA receptors are expressed in the rat nucleus accumbens (NAc) and functional KA receptors have been shown to be localized both pre- and post-synaptically (Takahata and Moghaddam, 2000; Crowder and Weiner, 2002). Additionally, ionotropic glutamate receptors are expressed on non-dopamine neurons in the ventral tegmental area (VTA) (Wang and French, 1995) and contribute to the functional integration of this system, as blockade of NMDA and AMPA/KA receptors has been shown to prevent the acquisition of D 2/3 dopamine receptor stimulation conditioned place preference (Suto et al., 2003; Biondo et al., 2005).

While increasing evidence emerges to support an important role for glutamate and its receptors in dopaminergic transmission, very little, to our knowledge, is known about the role of such receptors in the development and maturation of this pathway. Our interest was to determine whether chronic administration of a low, sub-convulsive dose of domoic acid (a selective KA receptor agonist), during a critical period of CNS maturation would alter the functional integration of the mesocorticolimbic pathway in the adolescent rat.

Materials and methods

Animals and experimental design

Within 24 h of birth, offspring of untimed-pregnant Sprague Dawley rats (Charles River Laboratories, St. Constant, PQ, Canada) were culled to 10–11 pups/litter (5 males and 5 females where possible). From PND 8–14, pups were weighed and given a single daily injection (s.c.; 10 ml/kg) of either saline ($n = 30$) or 20 μg/kg DOM ($n = 30$). This dose has been previously shown to be well below those normally required to induce overt toxicity in animals of this age (Doucette et al., 2000, 2003). Pups were weaned at 21 days of age (PND 21) with two to three rats (of same gender) per cage. All animals were housed within polypropylene caging with wood chip bedding, and the colony room was maintained at 22.2 °C with a 12 h light/dark cycle (07:00–19:00 h) with food (Purina Lab Chow) and water provided ad libitum.

Nicotine-induced conditioned place preference (late adolescence)

(−)-Nicotine hydrogen tartrate salt (obtained from Sigma, Canada) was diluted in physiological saline (1 ml/kg) for a dose of 0.6 mg/kg, and was adjusted to pH 7.0 using NaOH. The conditioned place preference (CPP)

chamber was constructed from 1/4 inch plexiglas with a removable partition in the center (i.e. which could divide the maze into two compartments of $41 \times 30.5 \times 46$ cm; each of which were visually and tactually distinct).

Testing involved an initial injection-habituation phase, a habituation trial, eight conditioning trials and lastly, a test trial. During the injection-habituation phase, rats received a single saline injection (s.c.) in the room in which the injections during conditioning would take place. This was done to habituate the rats to the injection procedure and reduce anxiety before the conditioning trials began. A habituation trial followed 24 h later, in which the rats were injected with saline, placed in the CPP maze with the door removed, and allowed access to both maze compartments for a total of 15 min. Conditioning trials began 24 h following the habituation trial and involved administering either saline or nicotine (s.c.; experimenter blind) to the rat and then placing it in the appropriate compartment for 30 min. Following this, the rat was removed from the maze and returned to the home cage for 24 h until the next trial, for a total of eight trials (four paired with nicotine and four with saline).

The procedure was an unbiased procedure, with half of the rats from each preweaning treatment (i.e. DOM and SAL) receiving nicotine always paired with one compartment, and the remaining rats receiving nicotine always paired with the other compartment. This was done to control for any biases that could exist in inherent preference for either of the two compartments. Thus, this was a within-subjects design with each rat receiving four pairings of nicotine and four pairings of saline, with drug (either nicotine or saline) on the initial conditioning day, counter balanced across conditions (DOM and SAL). Each day the pairing was alternated. After the eight conditioning trials, the rats were then placed in the CPP maze again for the test trial.

The test trial, in which the rat was placed in the middle of the maze, with the door removed, and allowed access to both compartments, was conducted 24 h after the last conditioning trial and was 15 min in duration. All testing was videotaped for later scoring, and amount of time spent in each compartment was scored.

Adherence to guidelines

All procedures were approved in advance by the UPEI Institutional Animal Care Committee and adhered to the guidelines of the Canadian Council on Animal Care.

Results

Nicotine induced conditioned place preference

SPSS (v. 11.2) was used to conduct paired samples *t*-tests, to compare the time (sec) spent in the nicotine paired (paired) compartment with the saline paired (unpaired) compartment in each of the treatment groups. Only the SAL males ($t(15) = 2.308$, $p = 0.036$) and SAL females ($t(13) = 2.508$, $p = 0.026$) demonstrated nicotine induced conditioned place preference (Fig. 1A and C). Interestingly, the DOM males and DOM females did not demonstrate conditioning (Fig. 1B and D). A two way ANOVA (treatment $\times$ gender) revealed no significant differences for number of center crosses, suggesting that there was no overall difference in general activity levels between groups.

Discussion

While investigation of the role of KA receptors to CNS physiology and pathology is a relatively new field of study, increasing evidence suggests a multitude of important physiological roles for KA receptors. For instance, recent literature suggests that KA receptors may play a significant role in modulating dopamine release within the mesocorticolimbic system (Mathé et al., 1998; Takahata and Moghaddam, 2000; Legault and Wise, 2001; Crowder and Weiner, 2002). It is our contention that KA receptors most likely also play a role in modulating behaviour controlled by this pathway. Thus, it would seem reasonable

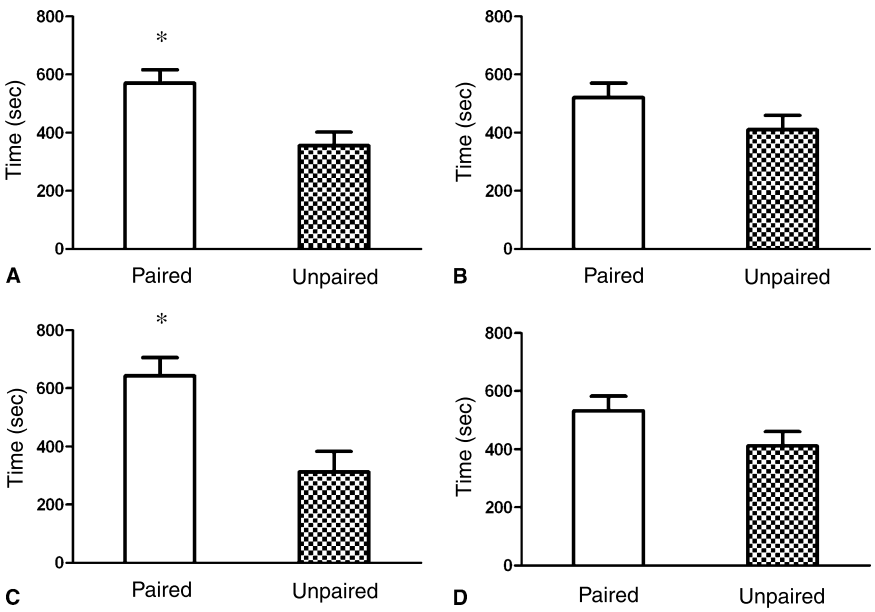

Fig. 1. Mean time (sec) spent in nicotine-paired and unpaired chambers in the 24 h following the final conditioning trial for male saline (**A**) and DOM-treated (**B**) rats and for female saline (**C**) and DOM-treated (**D**) rats. Error bars represent standard errors ($^*p < 0.05$ relative to unpaired compartment)

to speculate that if KA receptors are important in the ontogeny of the dopaminergic ''reward'' pathway, then mild and transient activity at KA receptors during the brain growth spurt should alter the functional integration of the mesocorticolimbic system.

The results of this study demonstrate that early exposure to low doses of DOM result in an altered response to reward during adolescence. This was illustrated by the DOM-treated rats failing to demonstrate a place conditioning preference to nicotine, which was present in the control animals. Adolescent rats are exquisitely sensitive to nicotine (Cruz et al., 2005) and readily condition to the drug under normal circumstances (Vastola et al., 2002). We not only demonstrated that the sensitivity to the conditioning properties of nicotine spans into late adolescence in the rat, but also that this sensitivity is altered in rats treated perinatally with low doses of DOM.

Rather than producing a transient pharmacological effect, receptor acting compounds administered to the immature organisms, especially during the brain growth spurt, have the potential to cause permanent, irreversible insult (Kaufmann, 2000). This insult can be expressed as either permanent dysfunction to the neurotransmitter system involved, or may result in ''irreversible imprinting'' of receptor densities, which in turn results in lasting functional and/or structural changes to the nervous system (Kaufmann, 2000). Thus, any number of possible mechanisms may underlie these behavioural alterations including alterations in regional receptor densities, synaptic transmitter concentrations, dopamine turn-over rates, susceptibility to nicotine induced synaptic plasticity, and may involved multiple transmitter systems (e.g. glutamate, GABA, dopamine, acetylcholine).

At this point it is unclear whether this decreased response is permanent or whether it reflects a transient lag in the developmental shifts in response to nicotine. However, our results do suggest that early exposure to DOM produces alterations in the ability of nicotine to induce a conditioned place preference in the adolescent rat, a developmental time period where the typical rat is exquisitely sensitive to the rewarding properties of nicotine (Vastola et al., 2002; Adriani et al., 2003; Cruz et al., 2005). These findings underscore the importance of glutamate (especially KA receptors) in the normal development of the CNS, specifically the mesocorticolimbic pathway.

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