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Hippocampal Y2 receptor-mediated mossy fiber plasticity is implicated in nicotine abstinence-related social anxiety-like behavior in an outbred rat model of the novelty-seeking phenotype

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ABSTRACT

Experimentally naïve outbred rats display varying rates of locomotor reactivity in response to the mild stress of a novel environment. Namely, some display high rates (HR) whereas some display low rates (LR) of locomotor reactivity. Previous reports from our laboratory show that HRs, but not LRs, develop locomotor sensitization to a low dose nicotine challenge and exhibit increased social anxiety-like behavior following chronic intermittent nicotine training. Moreover, the hippocampus, specifically hippocampal Y2 receptor (Y2R)-mediated neuropeptide Y signaling is implicated in these nicotine-induced behavioral effects observed in HRs. The present study examines the structural substrates of the expression of locomotor sensitization to a low dose nicotine challenge and associated social anxiety-like behavior following chronic intermittent nicotine exposure during adolescence in the LRHR hippocampi. Our data showed that the expression of locomotor sensitization to the low dose nicotine challenge and the increase in social anxiety-like behavior were accompanied by an increase in mossy fiber terminal field size, as well as an increase in spinophilin mRNA levels in the hippocampus in nicotine pre-trained HRs compared to saline pre-trained controls. Furthermore, a novel, selective Y2R antagonist administered systemically during 1 wk of abstinence reversed the behavioral, molecular and neuromorphological effects observed in nicotine-exposed HRs. These results suggest that nicotine-induced neuroplasticity within the hippocampus may regulate abstinence-related negative affect in HRs, and implicate hippocampal Y2R in vulnerability to the behavioral and neuroplastic effects of nicotine in the novelty-seeking phenotype.

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1. Introduction

Individual differences in the degree to which humans voluntarily participate in activities associated with personal risk (Zuckerman, 1984) positively correlate with drug addiction and other psychopathologies (Zuckerman and Neeb, 1979). The novelty-seeking phenotype (Piazza et al., 1989; Hooks et al., 1991) was originally introduced as an animal model that overlaps with the human sensation-seeking trait (Zuckerman, 1984), and is identified in the laboratory rat by assessing locomotor reactivity to the mild stress of a novel environment. Namely, some rats display high locomotor reactivity in novel environments and are identified as high responders (HRs), whereas some display low locomotor behavior in novel environments and are identified as low responders (LRs). We have previously shown that a behaviorally-sensitizing nicotine regimen results in increased suprapyramidal mossy fiber (SP-MF) terminal field size in the HR but not LR rats (Bhatti et al., 2007). Moreover, temporarily inactivating the hippocampal hilus region results in augmented locomotor response to challenge nicotine in HRs, implicating MF plasticity in the behaviorally-sensitizing effects of nicotine (Bhatti et al., 2007).

The hippocampal MF comprise the axons of the dentate gyrus granule neurons that innervate the CA3 field (Gaarskjaer, 1986). Morpho-behavioral correlations in genetically-altered mice implicate hippocampal MF with exploratory behavior (Roullet and Lassalle, 1990; Ivanco and Greenough, 2002; Mineur et al., 2002). Moreover, data from inbred mice correlate heritable variance in MF size with different emotional behaviors, such as fear conditioning and anxiety-like behavior (Prior et al., 1997). Likewise, studies on outbred rats show a positive correlation between MF sprouting and anxiety-like behavior in the light–dark box test (de Oliveira et al., 2008), implicating MF plasticity in the regulation of anxiety.

We have recently shown an upregulation in Y2 receptor (Y2R) and a downregulation in neuropeptide Y (NPY) mRNA levels in the hippocampal CA3 field in HRs following a behaviorally-sensitizing nicotine regimen (Aydin et al., 2011a,b). Y2R-specific mechanism of NPY binding is implicated in the MF system following prolonged seizures, where NPY reduces glutamate release by activating presynaptic Y2R in MF pathway

Abbreviations: HR, high responder; IIP, intra- and infra-pyramidal; LR, low responder; MF, mossy fiber; NPY, neuropeptide Y; SP, supra-pyramidal; SI, social interaction; Y2R, Y2 receptor.

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(Nadler et al., 2007), suggesting a regulatory role for NPY via Y2R in the hippocampal MF system. Incidentally, NPY in the hippocampus has been reported to have anxiolytic properties (Thorsell et al., 2000; Lin et al., 2010; Cohen et al., 2011). Indeed, reversal of the deficit in hippocampal NPY mRNA levels in nicotine pre-trained HRs by systemic injections of a Y2R antagonist, JNJ-31020028 during 1 wk of abstinence accompanies reversal of the increased anxiety-like behavior in these animals (Aydin et al., 2011b), confirming the regulatory role of hippocampal NPY in anxiety-like responses. On the other hand, whether Y2R-mediated modulation of the MF system alters anxiety-like behavior remains to be answered.

The present study investigated the efficacy of a systemically administered Y2R antagonist on reversing nicotine-induced MF sprouting and the increase in social anxiety-like behavior observed in HRs following a behaviorally-sensitizing nicotine regimen. We also determined whether nicotine-induced MF sprouting observed in HRs was reflected on the CA3 dendritic receptive fields by assessing mRNA levels of a postsynaptic marker, spinophilin that is preferentially located in dendritic spines (Allen et al., 1997), and whether Y2R antagonist treatment altered these nicotine-induced neuroplastic effects.

2. Materials and methods

2.1. Drugs

Nicotine hydrogen tartrate was obtained from a commercial supplier (Sigma), dissolved in 0.9% NaCl and the pH was adjusted to 7.4. The Y2R antagonist, JNJ-31020028, was generously donated by Janssen Research & Development, L.L.C., and dissolved in 20% 2-hydroxypropyl-beta-cyclodextrin. The doses for nicotine and JNJ-31020028 were chosen based on effective doses used in the literature in several reports by others (Miller et al., 2001; Suto et al., 2001; Shoblock et al., 2010), and by us (Bhatti et al., 2007, 2009; Aydin et al., 2011a,b, 2012a).

2.2. Animal housing and the LRHR phenotype screening

Animals were treated in accordance with the National Institute of Health guidelines on laboratory animal use and care. All efforts were made to minimize animal suffering and to reduce the number of animals used. A grand total of 72 male Sprague–Dawley rats (Charles River, Wilmington, MA) arrived at weaning (postnatal day, PN 22), were housed 3 per cage in 43 × 21.5 × 25 cm^3 Plexiglas cages and were kept on a 12 h light/dark cycle (lights on at 7:00 A.M.). Food and water were available ad libitum. Animals were allowed to habituate to the housing conditions and were handled daily for 2 days. On PN 25, animals were screened for locomotor reactivity to the mild stress of a novel environment for 1 h using commercially available locomotion chambers (San Diego Instruments, San Diego, CA). Briefly, locomotor reactivity to novelty was tested in 43 × 43 × 24.5-cm^3 (high) clear Plexiglas cages with stainless steel grid flooring. Activity was monitored by means of photocells (a total of X = 16 by Y = 16 photocells) 2.5 cm above the grid floor and equally spaced along the sides of the box. Horizontal locomotion was monitored by this lower bank of photocells. Each locomotor count recorded a minimum of 3-cm traversing of the cage. Additional photocells were located 11.5 cm above the grid floor and 9 cm above the lower bank of photocells. Rearing (i.e., locomotion in the Z plane) was monitored by this upper bank of photocells. At the end of the screening session, total locomotor activity (i.e., X, Y, and Z locomotion) was pooled and the rats were ranked as HRs (i.e., rats that exhibited locomotor scores in the highest third of the sample; n = 24) or LRs (i.e., rats that exhibited locomotor scores in the lowest third of the sample n = 24). The intermediary responders were only used as resident rats in the social interaction test described below.

2.3. Behavioral sensitization to nicotine and therapeutics administration

The behavioral sensitization to nicotine procedure is outlined in Table 1. Male Sprague–Dawley rats (PN 22) were allowed to rest until PN 28 after phenotype screening, and were assigned to saline (1 ml/kg; s.c.) or nicotine (0.35 mg/kg; s.c.) training injection groups. On injection days, rats were given 1 h to habituate to the locomotor chambers before they received an injection of the assigned drug. Their locomotor response was recorded for 90 min. This procedure was repeated four times at a 3-day interval. Following the fourth training injection, rats were further assigned to vehicle (1 ml/kg, i.p.) or JNJ-31020028 (20 mg/kg, i.p.) therapeutic injection groups, and underwent 1 wk of abstinence where they received daily vehicle or JNJ-31020028 injections. At the end of the abstinence period, all LRHR rats were challenged with a low dose of nicotine (0.1 mg/kg, s.c.), and their locomotor response was monitored for 45 min. Upon completion of the challenge session, rats were tested on the social interaction test for assessing anxiety-like behavioral profile as described below.

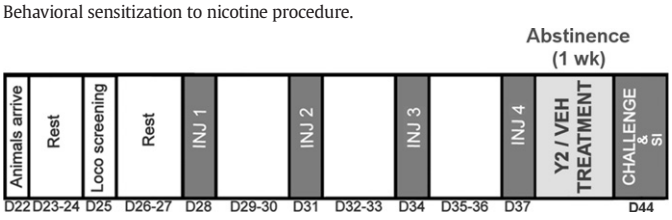
2.4. Social interaction test (SI)

Testing took place in an open topped, rectangular, transparent social interaction box. The resident rat was placed in the box 8 min prior to placement of the experimental rat. The resident rat was of similar weight that was housed under identical conditions as the experimental rat, which had no previous contact with the experimental rat. The experimental rat was placed in the box and the amount of time the experimental rat spent initiating social interaction (i.e., grooming, sniffing, following, and crawling over or under) with the resident was determined for 5 min. Upon completion of testing, animals were rapidly decapitated, brain tissues were harvested and processed for in situ hybridization histochemistry and Timm's method for silver sulfide staining as described below.

2.5. In situ hybridization histochemistry

Brain tissues were collected and immediately frozen in isopentane cooled to −30 °C. Brains were then sectioned on a cryostat and 20-μm-thick coronal sections were mounted on electrostatically charged slides. These slides were kept at −80 °C until processed. On the day of hybridization, sections were fixed in 4% paraformaldehyde at room temperature for 1 h, followed by three washes in 2× SSC (1× SSC is 150 mM sodium chloride, 15 mM sodium citrate). Sections were placed in a solution containing acetic anhydride (0.25%) in triethanolamine (0.1 M, pH 8) for 10 min, rinsed in distilled water, dehydrated through graded alcohols (50%, 75%, 85%, 95% and 100%) and air-dried. Rat spinophilin cDNA was cloned in our laboratory (accession #: NM_053474), antisense linearized, transcribed, and ^{35}S labeled in reaction mixtures consisting of 1 ml of linearized plasmid, 1× transcription

Table 1
Behavioral sensitization to nicotine procedure.



Phenotype pre-screened LRHR rats received saline (1 ml/kg; s.c.) or nicotine (0.35 mg/kg; s.c.) injections four times at 3-day intervals (PN 28–37). Following the last saline or nicotine injection, all animals underwent 1 wk of abstinence during which they received daily, systemic Y2R antagonist (20 mg/kg; i.p.) or vehicle (1 ml/kg; i.p.) injections. At the end of the abstinence period (PN 45), all animals were challenged with a low dose of nicotine (0.1 mg/kg; s.c.).