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Josie Minnigh

East Tennessee State University

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BRAIN-DERIVED NEUROTROPHIC FACTOR LEVELS IN D2 RECEPTOR
PRIMED ADOLESCENT RATS GIVEN TWICE DAILY NICOTINE
ADMINISTRATIONS

Thesis submitted in partial fulfillment of Honors

By

Josie Minnigh

The Honors College

Midway Honors Program

East Tennessee State University

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Russell Brown, Faculty Mentor

Josie Minnigh, Midway Honors Scholar

Abstract

Nicotine use is very prevalent in the schizophrenic population, which is a 2.5 fold greater than the general population. In the present study, the drug quinpirole (dopamine D2/D3 agonist) or saline was given neonatally to 25 Sprague-Dawley male and female rats. Rats were randomly assigned to condition. Beginning postnatal day 33 animals were given twice daily administrations of nicotine (0.5 mg/kg free base). After the first of the daily injections they were placed in a locomotor arena every other day for behavioral testing. One day after behavioral testing, the dorsal striatum and nucleus accumbens were removed for brain-derived neurotrophic factor (BDNF) assay. BDNF is a neurotrophin that plays an important role in neuronal development, neuronal maintenance and plasticity, and synaptic activity. Results showed that nicotine produced locomotor sensitization but this was not enhanced by neonatal quinpirole, unlike past work. Regarding BDNF, there was a significant increase in the nucleus accumbens in rats treated with nicotine; neonatal quinpirole increased the BDNF response produced by nicotine. Nicotine produced an increase in dorsal striatum BDNF that was not affected by neonatal quinpirole treatment. Importantly, it appears that nicotine administrations, that occurred in two different contexts, may result in differential behavioral results relative to nicotine administrations given consistently in the same context.

Introduction

Schizophrenia is a mental disorder that can be severely debilitating. It affects as much as 1% of the population and is a common disorder seen in institutions today (Asher & Gask, 2010). As stated in the DSM-IV TR, to be clinically diagnosed with schizophrenia, one must have at least two of the following symptoms for at least one month: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, and negative symptoms (American Psychiatric Association, 2000).

Negative symptoms in schizophrenia are defined as “weakening or lack of normal thoughts, emotions or behavior” (Makinen, Miettunen, Isohanni, & Koponen, 2008, p. 334). One in three people diagnosed with schizophrenia has negative symptoms (Makinen et al., p. 334). Positive symptoms include delusions, hallucinations, thought disorders, and movement disorders. These symptoms prevent the person from functioning as they do normally. They put stress on relationships with family and friends, their occupations, academics, etc. These must last for a six month period with at least one month of the previously mentioned symptoms.

A common problem observed in schizophrenic patients is substance abuse. Ringen et al. (as cited in Asher & Gask, 2010, p. 1) reported that “11.9% of the people with schizophrenia comorbid drug abuse or dependence.” There are many theories on why this is so. Drugs that are commonly abused are nicotine, alcohol, and cannabis. By studying substance abuse in schizophrenic patients, which has devastating health consequences, methods can be formed to prevent them from dying at such an early age (Kotov, Guey, Bromet, & Schwartz, 2010).

In a study completed by Leon and Diaz (as cited in Kotov et al., 2010) they estimated that 62% of the schizophrenic population smokes. This raised the question by Kotov, Guey,

Bromet, and Schwartz (2010), whether there was a correlation between schizophrenia and smoking. They took a sample size of 542 patients and followed them for 10 years after their first hospitalization. The authors also completed 5 interviews with the patient. The average number of cigarettes smoked per day was 16.1. The findings showed a 2.5 fold greater use than the U.S. population. Smoking increased during periods of depression (Kotov et al., 2010). Many studies have been conducted in order to better understand the correlation of nicotine abuse in the schizophrenic population.

Previously in the lab, we have shown that neonatal treatment with the dopamine D2/D3 agonist quinpirole results in long-term increases in dopamine D2 receptor sensitivity, a phenomenon known as 'D2 Priming. Neonatal quinpirole treatment also produces several different abnormal behaviors and cognitive impairments similar to schizophrenia (Brown, Perna, Schaefer, & Williams, 2006). Neonatal quinpirole also results in decreased neurotrophins in the hippocampus and frontal cortex, such as BDNF, which may play a role in long term cognitive effects due to increased activity at the D₂ receptor (Brown, Flanigan, Thompson, Thacker, Schaefer, & Williams, 2004).

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays an important role in neuronal development, neuronal maintenance and plasticity, and synaptic activity (Brown et al., 2006). It is the most widely distributed neurotrophic factor found in the central nervous system. BDNF is found in the hippocampus and prefrontal cortex, which are areas in the brain that mainly function in cognitive performance (Brown et al., 2006). Several studies have shown that nicotine produces an increase in neurotrophins in several different brain areas (Kenny et al., 2000; French, et al., 1999). Multiple research studies have linked a correlation in involvement of BDNF in the pathophysiology of schizophrenia. Positive and Negative Syndrome Scale

(PANSS) symptoms have been shown to lower in schizophrenics who smoked compared to schizophrenics who did not smoke and BDNF levels were higher in the smokers than in the nonsmokers (Zhang, Xiu, Chen, Yang, Wu, Lu, Kosten, & Kosten, 2010). It has been suggested that this change may be associated with nicotine receptor-induced upregulation of BDNF (Zhang et al., 2010). Based on these findings, schizophrenics have decreased BDNF levels compared to the general population. Nicotine increases these BDNF levels. It is hypothesized that there will be an increase in BDNF in the rats that are administered nicotine.

Materials and Methods

A total of 25 Sprague-Dawley rats were raised in our animal colony. These animals were the offspring of 5 pregnant females acquired from Harlan, Inc. (Indianapolis, IN). A total of 14 females and 11 males were used as subjects in this study. They were categorized into 4 groups depending on neonatal drug (Quinpirole = Q; Saline = S) and adolescent drug (Nicotine = N; Saline = S) treatment: SN= 7, SS= 6, QN= 6, and QS=6. The day of birth is referred to as postnatal day (P) zero or P0. Rats were injected with quinpirole (1mg/kg) or saline, intraperitoneally, on P1 through P21, through random assignment. P21 marked when they were weaned from the female dam and were raised to P29. The subjects were then housed 2-4 rats per cage. The adolescent rats lived in a room that was climate-controlled with a 12 hour light-dark cycle.

At P29 the rats were habituated to the locomotor arena. Habituation is used so that the rats get repeated exposure to the injections and locomotor arena so that the behavioral responses are not due to sensory adaptation or motor fatigue. They were injected with saline from P29 to P31, and then placed in the locomotor arena for 10 minutes, 10 minutes post injection. Nicotine

sensitization began at P33 and ended at P49. The subjects were injected twice daily with saline or nicotine (0.5 mg/kg), i.p. in the morning and afternoon on each day. This represents a dose that was 4 times what we had previously administered to adolescent rats. They were tested every other day in the locomotor arena for a total of 9 days. Rats were placed in the locomotor arena ten minutes after they were injected i.p. with either nicotine or saline. The locomotor arena consisted of four wooden boxes placed next to each other that were painted flat black. They measured 40 cm on each side. One rat was placed in each box so that four rats could be recorded at a time. A digital camera was placed above the locomotor arena, which was connected to Any Maze (Stoelting, Wood Dale, IL) tracking system software. This software measured behavior by keeping track of horizontal activity. The software program places a digital grid with 5 cm x 5cm squares. Each time the subject crosses the line it counts as horizontal activity.

The brain tissue was taken on P50 to be analyzed for BDNF levels. The brain areas that were dissected were the dorsal striatum and the nucleus accumbens. These specific areas are where dopaminergic pathways run and where decrease levels of BDNF occur in individuals with schizophrenia. In the accumbal area, nicotine is known to increase levels of BDNF (Correll et al., 2009). These brain areas were also studied because neonatal quinpirole treatment results in a significant decrease in G-protein signaling (RGS), specifically Rgs9, in the striatum, nucleus accumbens, and frontal cortex (Maple, Perna, Parlaman, Stanwood, & Brown, 2007). This decrease in RGS is similar to findings observed in postmortem samples of striatum in schizophrenic individuals (Seeman et al., 2006).

Results

A 2 x 2 x 2 ANOVA was used for statistical analysis. Activity counts are presented as a function of day in Figure 1 A 2 (neonatal drug treatment) x 2 (adolescent drug treatment) x 2 (day of testing) revealed a significant main effect of adolescent drug treatment $F(1,20) = 9.74$, $p < .005$, day of testing $F(1,20) = 128.28$, $p < .001$ and a significant two-way interaction of adolescent drug treatment x day of testing $F(1,20) = 41.10$, $p < .001$. Nicotine produced an increase in locomotor activity on day 9, but there were no effects on day 1. Importantly, neonatal quinpirole did not enhance sensitization to nicotine at day 9.

BDNF is presented as a function of drug group in Figure 2 for dorsal striatum (left column) and nucleus accumbens (right right column). For the striatum, a 2 (neonatal drug treatment) x 2 (adolescent drug treatment) x 2 (day of testing) revealed a significant main effect of adolescent drug treatment $F(1,20) = 5.41$, $p < .032$. Nicotine produced an increase in striatal BDNF that was not affected by neonatal quinpirole treatment.

For the nucleus accumbens, a 2 (neonatal drug treatment) x 2 (adolescent drug treatment) x 2 (day of testing) revealed a significant main effect of neonatal $F(1,20) = 13.15$, $p < .002$ and adolescent drug treatment $F(1,20) = 16.65$, $p < .001$. Both neonatal quinpirole and adolescent nicotine produced a significant increase of BDNF in the nucleus accumbens. An independent t-test revealed a significant increase in Group QN as compared to Group SN ($p < .001$). Therefore, it appears neonatal quinpirole increased the BDNF response produced by nicotine.

Discussion

Findings revealed that the behavioral data of locomotor sensitization showed there was no effect on day 1, but nicotine did produce an increase in activity on day 9. The results on figure

1 show that neonatal quinpirole did not enhance nicotine sensitization at day 9. This may be due to the fact that nicotine was administered once every other day in the locomotor arena, but was administered at their cage the other 3 times. Since adolescents were receiving the drug's rewarding effects at their cage more often than the locomotor arena, this may have resulted in a reduced behavior effect of nicotine in rats neonatally treated with quinpirole. Studies have shown that nicotine is known to have strong associations with non-reinforcing cues (Palmatier, O'Brien, & Hall, 2011). Based on these data, this would suggest that nicotine's association with non-reinforcing cues may alter the behavioral effects in neonatal quinpirole treated animals, yet not block sensitization altogether. Neurochemical findings showed that nicotine altered BDNF levels in quinpirole treated rats. In the nucleus accumbens, there was an increased BDNF response in the QN group when compared to the SN group. This is consistent with previous findings in this laboratory (Correll, Noel, Sheppard, Thompson, Li, Yin, & Brown, 2009). However, BDNF levels were not affected by neonatal quinpirole treatment when looking at the dorsal striatum results. By analyzing figure 2, the nicotine did produce an increase in BDNF, but it was not related to neonatal quinpirole.

As mentioned previously, schizophrenic individuals have decreased BDNF levels. Nicotine increases BDNF levels, which decreases some of the abnormal behavioral effects brought on by this disorder. In a study completed by Tizabi, Copelland, Brus, & Kostrzewa (1999), it was observed that nicotine is able to block some of the behavior effects caused by quinpirole. This is directly related to the nicotinic receptor antagonist, mecamylamine, in which there is an increase of receptor binding. These results suggest that nicotinic agonists may shine light in possible therapeutic agents for disorders related to the dopamine system (Tizabi et al., 1999). However, with increased BDNF levels observed in regions of the brain due to nicotine

treatment, it suggests that nicotine is involved in changes in neural development and maintenance. With much change observed in adolescent brain development, there could be serious consequences. The increased amount of smoking seen in the schizophrenic population is a health hazard and is causing premature deaths. It is important to try and break the habit of smoking in adolescents with this disorder.

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Figures

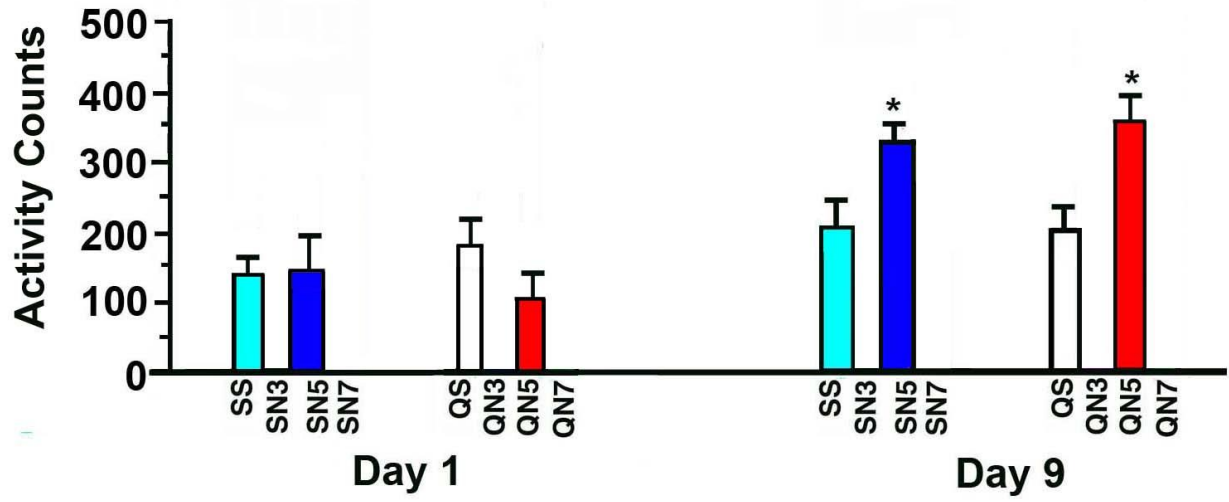


Figure 1

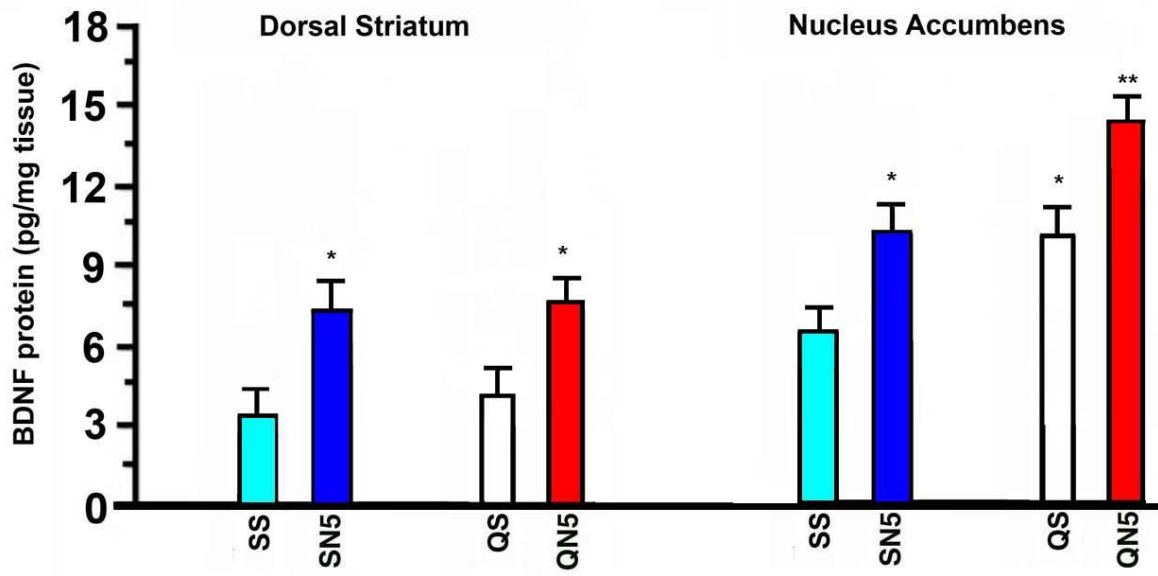


Figure 2