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Altered pre-pulse inhibition in adult rats treated neonatally with domoic acid

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Summary. Altered functioning of the glutamate system during critical periods of development is believed to play a role in various neurodevelopmental disorders, such as schizophrenia. Prepulse inhibition (PPI) of the acoustic startle response is deficient in people with schizophrenia. This study investigated the theory that neonatal treatment with domoic acid (DOM), a glutamate agonist, leads to deficient PPI. Results indicate that neonatal treatment with DOM leads to lowered PPI in adult males and an increased startle response in adult females.

Keywords: Kainate receptors – Glutamate – Brain development – Pre-pulse inhibition – Schizophrenia

Introduction

Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system (Ozawa et al., 1998). It is suspected that abnormal glutamate functioning during critical periods of development may be a contributing factor in various neurodevelopmental disorders such as schizophrenia. Altered functioning of the glutamate system during these periods can have long-lasting effects on the developing central nervous system (McDonald and Johnston, 1990).

Our current and past research has focused on how neonatal treatment with low, sub-convulsant doses of DOM (a kainate receptor agonist) affects behavior in adulthood. Theoretically, this treatment with DOM results in the glutamate system being activated above the level that would be normal for this developmental stage. Results have shown abnormalities in seizure-like behavior, altered responses to novelty, changes in cognitive functioning and altered neuroanatomy (i.e. the hippocampus) (Doucette et al., 2003, 2004, 2007; Tasker et al., 2005).

While the exact etiology of schizophrenia is not yet known, abnormal glutamate receptor functioning has been linked to this neurodevelopmental disorder (Stone et al., 2007). Additionally, altered cognitive functioning, changes in response to novelty, epilepsy comorbidity and hippocampal changes are consistent with both clinical manifestations of schizophrenia and with the changes that result following neonatal rodent exposure to DOM. Taken together, this pattern of anomalies suggests that early overactivation of the glutamate system during a critical period of development is a possible animal model of schizophrenia.

Animal models provide one of the best ways to study the underlying neurobiological mechanisms that contribute to a human disorder such as schizophrenia. Current animal models of schizophrenia are helpful, but far from perfect. To gain a better understanding of this disorder, it is essential to improve upon current models, as well as to pursue evidence of new models which may lead to further breakthroughs.

An ongoing issue in the development of animal models of neuropsychiatric disorders such as schizophrenia, is the difficulty in finding common symptoms which are displayed in various species. For this reason, relatively simple behaviors such as reflexes, often provide invaluable information regarding the suitability of certain animal models. Prepulse inhibition of the acoustic startle response is one such behavioral measure. Observed in many different species, including rats and humans, PPI is the normal suppression of the startle reflex that occurs when the startling stimulus is preceded by a less intense, non-startling stimulus (Graham, 1975). Believed to be controlled by

structures located in the lower brainstem and mediated by input from the forebrain (Weiss and Feldon, 2001), PPI is reliably disrupted in humans with schizophrenia (Braff et al., 1978) and has become widely used as a requisite symptom in studies of the neural effects of schizophrenia as well as in the search for useful animal models of the disorder (Swerdlow and Geyer, 1998; Swerdlow et al., 2000; Weiss and Feldon, 2001; Van den Buuse, 2005).

The current study investigated the possible relevance of early overactivation of the glutamate system as an animal model for schizophrenia by determining if rats treated neonatally with low doses of DOM show deficits in PPI as adults.

Materials and methods

Animals ($n = 45$) were born in house and given a single daily subcutaneous injection of either saline or 20 μg/kg of DOM (BioVectra DCL, Charlottetown, PEI, Canada) from postnatal day (PND) 8–14, following the procedure outlined in Doucette et al. (2007). Animals were maintained under standard laboratory conditions with testing beginning at PND 90. All testing procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

The startle apparatus was an SR-Lab from San Diego Instruments (San Diego, CA, U.S.A.). All animals received a 5 min acclimation period to the chamber before the experiment, which consisted of 3 blocks of trials, began. The intertrial interval for all trials was an average of 15 sec (ranging from 10 to 20 sec) and a background white noise level of 70 dBs was maintained. Average startle amplitude was obtained by measuring every 1 msec for 100 msec after the onset of the startle pulse, startle amplitude is defined as the average of the 100 readings.

Blocks 1 and 3 consisted of 5–6 120 dBs white noise startle pulses, each 40 msec in length. These trials were used to normalize startle, to establish a baseline for the animal's individual startle response at the beginning and end of the session, to determine if there was any difference between the two groups in their startle amplitudes independent of PPI, and to measure within-test habituation. The data from these trials was not included in the calculation of %PPI.

The %PPI procedure (block 2) was adapted from Pietraszek et al. (2005) using prepulse levels of either 4, 8, 12, or 16 dBs above the background noise. The %PPI was calculated by the following formula: $\text{PPI} = 100 - (\text{P}/\text{S}) * 100$, where P is the average startle amplitude for prepulse-pulse trials and S is the average startle amplitude on startle pulse alone trials.

Results

Analysis of the %PPI in males produced a significant main effect for condition at 82dBs [$t(21) = -1.804$, $p = 0.043$], with the DOM treated animals displaying lower PPI than the saline treated animals (Fig. 1A). Although no significant effects were found at the other prepulse levels, a consistent trend of lowered PPI in the DOM treated rats was shown. A comparison of the movement of the animals during testing showed that there was no significant differences in the overall movement of the male animals. Additionally, there were no group differences in the base-

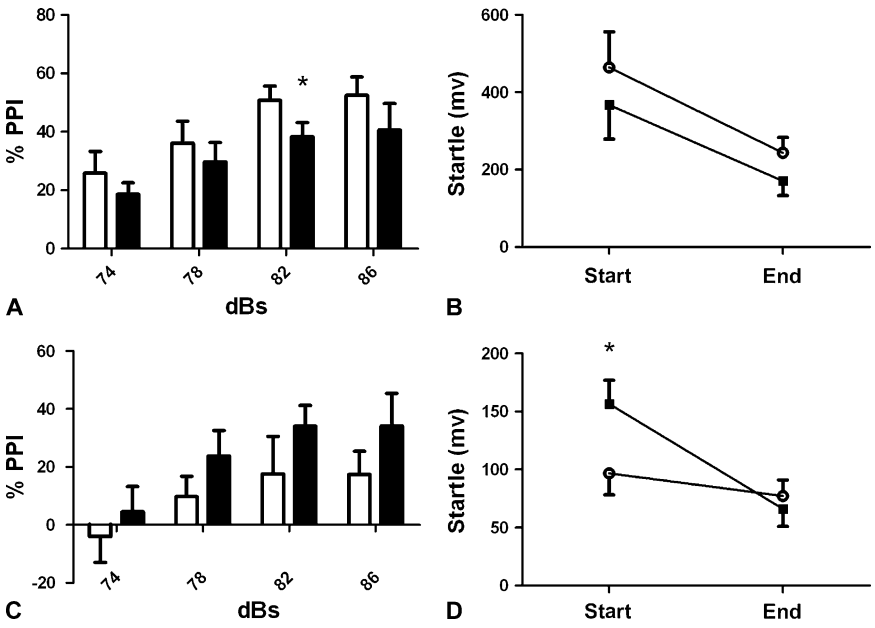

Fig. 1. (**A**) and (**C**) indicate the mean ± SEM %PPI at different prepulse decibel levels for (**A**) male SAL ($n = 11$) (*open bar*) and DOM ($n = 12$) (*solid bar*) treatment groups, and (**C**) female SAL ($n = 12$) (*open bar*) and DOM ($n = 10$) (*solid bar*) treatment groups. The asterisk indicates a significant difference from saline controls for that decibel level. (**B**) and (**D**) display the mean ± SEM startle amplitude at the beginning and end of the session for (**B**) male SAL ($n = 11$) (*open circle*) and DOM ($n = 12$) (*solid square*) treatment groups, and (**D**) female SAL ($n = 12$) (*open circle*) and DOM ($n = 10$) (*solid square*) treatment groups. The *asterisk* indicates a significant difference from saline controls for that period. Errors bars represent SEM for each time period

line startle level of the male rats before PPI testing began, or after the session ended (Fig. 1B). These results are supported by the finding of no difference between the habituation scores of the two groups during testing.

Analysis of the %PPI in the female animals produced no significant effect for condition at any prepulse level (Fig. 1C). Further investigation revealed that there was a significant difference in the females with regard to their baseline startle response at the beginning of the session [$t(20) = 2.177$, $p = 0.021$], with DOM treated females displaying a significantly higher average startle than saline treated females, although no difference was found at the end of the session (Fig. 1D). Additionally, a significant difference was found between the habituation scores of the two groups [$t(10.965) = 2.394$, $p = 0.018$] with the DOM treated females displaying a significant decline in startle response during testing, as compared to the saline treated females. No differences in movement during testing were detected.

Discussion

The results of this study show that the acoustic startle response and PPI are affected by neonatal treatment with DOM. The data indicates that neonatal treatment with subconvulsant doses of DOM leads to lowered PPI in adult male rats at the 82 dBs prepulse level. This significant difference between the groups is further supported by the fact that the animals did not differ in baseline startle response before or after testing, nor did they show differential movement or habituation during testing. These finding indicate that not only did the male DOM treated rats display deficient levels of PPI, but that these changes can not be attributed to differences in the groups' initial startle behavior.

Female rats showed no significant group differences in PPI. This may, in part, be due to the large variability in %PPI displayed in this study. However, the baseline startle levels of the two groups were significantly different, indicating that female rats, treated neonatally with DOM, display a heightened startle response. At the end of testing however, when startle response was measured again, there was no difference between the DOM and saline treated females, indicating that although their startle response was different in the beginning, by the end of testing it was equal to that of their saline counterparts.

Differences in startle response and PPI between males and females have been displayed in both humans (Kumari et al., 2004) and rats (Lehmann et al., 1999), with males consistently displaying greater PPI, particularly at lower prepulse levels. It is therefore possible that differences in startle between males and females contributed to the variability in %PPI between sexes found in this study.

The acoustic startle response may also be affected by the arousal of the animal. While Weiss et al. (1999) did not find any differences in the startle amplitude of animals tested during the light or dark phase of their cycle, others have found a significant increase in startle when testing during the dark cycle (Chabot and Taylor, 1992; Frankland and Ralph, 1995). Further research in this lab has shown a significant increase in %PPI (averaging 30%) in female untreated rats at the 86 dBs level when tested during the dark phase vs. the light phase of the cycle in an unlit chamber (unpublished data, 2007). Further testing of this animal model during a different time of day may lead to greater stability of the startle reflex and perhaps provide more consistent results.

In conclusion, we have shown that neonatal treatment with DOM produces PPI deficits in adult male rats as well as an initial heightened acoustic startle response in females. These finding support further investigation of this treatment as a potential animal model of schizophrenia.

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