

PRENATAL NICOTINE EXPOSURE AND THE 5HT α -7 RECEPTOR

By

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Abstract

Prenatal nicotine exposure is a known risk factor for Sudden Infant Death Syndrome (SIDS). This risk increase is thought to be the result of developmental nicotine exposure (DNE) affecting the development of the brain's control of breathing. This experiment was executed to determine what effect, if any, this type of nicotine exposure would have on the serotonin (5HT) system, specifically the 5HT α -7 receptor. Prenatal rat pups were exposed to nicotine using osmotic mini-pumps during pregnancy, with continued exposure through breast milk after birth (DNE). Brainstem-spinal cord preparations from 1-5 day old pups, both DNE and an unexposed control group, were placed into a split-bath and drugs were administered to either the brainstem or the spinal cord. The frequency and amplitude of action potential bursts were measured in the brainstem (rostral) experiments and tonic activity was measured in the spinal cord (caudal) experiments. Both experiments followed the same drug application protocol. A baseline of ten minutes was established, followed by fifteen minutes of 5HT α -7 antagonist application, followed by thirty minutes of 5HT α -7 antagonist + 5HT application, followed by washout for twenty minutes. DNE exposure had statistically significant effects in the rostral experiments, but produced non-significant trends in the caudal experiments.

Introduction

Sudden infant death syndrome (SIDS) is defined as when a healthy infant dies unexpectedly while they are sleeping (5). These infants are under one year old and do not exhibit any outward signs of poor health or obvious cause of death. SIDS is the leading cause of death in the United States for infants between 1 and 12 months old (4). While the mechanism of SIDS is unknown, one plausible theory is that the infant affected has some abnormality in the areas of the brain that control breathing and heart rate. These abnormalities can then lead to apnea and

reduced cardiac output, causing the infant to stop breathing during sleep. SIDS follows the triple-risk model, which states that SIDS occurs when an infant is exposed to three overlapping risk factors at the same time (2). These three risk factors are having a vulnerable infant, i.e. one that has some type of genetic predisposition or brain abnormality that puts them at risk for SIDS, that infant being in the critical developmental period, i.e. under the age of one year old, and that infant being exposed to an outside stressor, i.e. a blanket covers their mouth or being placed on their stomach to sleep. The outside stressor results in the infant breathing in a higher concentration of carbon dioxide and a lower concentration of oxygen while they sleep. Smoking during pregnancy has been strongly associated with SIDS and is considered to be one of the major risk factors that result in a vulnerable infant (1).

The role of serotonin in the control of breathing is any area of science that is still not understood very well. Serotonin receptors and projections from serotonin neurons are located all throughout the respiratory network, which has led scientists to believe that serotonin must have an active role in breathing regulation, especially in neonates (3). Another hypothesized that the serotonin system could be responsible for chemosensitive in the control of breathing, due to small, but prominent population of serotonin neurons that are embedded in the arcuate nucleus (4). Dysfunction in the peripheral chemosensitive system would result in an inability for an infant to accurately detect rising levels of CO₂ in the blood stream. This means that if an infant with this defect were faced with a breathing challenge, they would be unable to respond adequately and would continue to rebreathe CO₂ until they passed away. An association between SIDS infants with serotonin abnormalities and those same infants being found in asphyxiating environments support this theory (4). It was also found when examining a group of American Indian woman that serotonin receptor binding was greatly reduced in infants whose mothers

smoked during pregnancy (4). This association points to nicotine exposure during pregnancy being the cause of these abnormalities in serotonin receptors. There are many different serotonin receptor types and the individual contributions of each type of receptor are not yet established. This thesis will be focusing on the 5HT alpha-7 receptor in order to determine what its contribution to breathing control is and how its contribution to the brainstem-spinal cord response to serotonin is affected by developmental nicotine exposure (DNE).

Methods

Animal Exposure

The animals used in these experiments were neonatal rats of either sex, ranging in age from day one after birth (P1) to day five after birth (P5). These rats were born via spontaneous vaginal delivery from either nicotine exposed (DNE) or unexposed (control) mothers. Neonates were housed with their mothers and siblings until each individual was used for experimentation. Rats were housed in a quiet room with a temperature of 21–23 °C, 20-30% relative humidity and 12/12h light/dark cycles. The mothers had unlimited access to food and water. Seven neonates from each group, control and DNE, were used for the rostral experiments and five neonates from each group were used for the caudal experiments. DNE mothers were implanted with subcutaneous osmotic mini-pumps on gestational day five. These pumps administered a dose of 6mg/kg/day of nicotine bitartrate in order to mimic the plasma cotinine levels found in the umbilical cord blood of infants whose mothers smoke heavily during pregnancy. Infusion persisted though out the entire pregnancy and seven days postnatal. The control group mothers were not implanted with mini-pumps any time during pregnancy.

Brainstem-Spinal Cord Preparation

Neonatal rats were placed on ice in order to be anesthetized via hypothermia. The Brain stem and spinal cord were removed as one unit, beginning at the pontomedullary junction and extending below the fourth cervical nerve roots. These preparations were then placed in a dissection dish in clean, artificial cerebral-spinal fluid (aCSF) and perfused with O₂ for thirty to forty-five minutes in order to allow the preparation to equilibrate to room temperature. The brain stem-spinal cord preparation was then placed in a split-bath configuration, see figure 1.

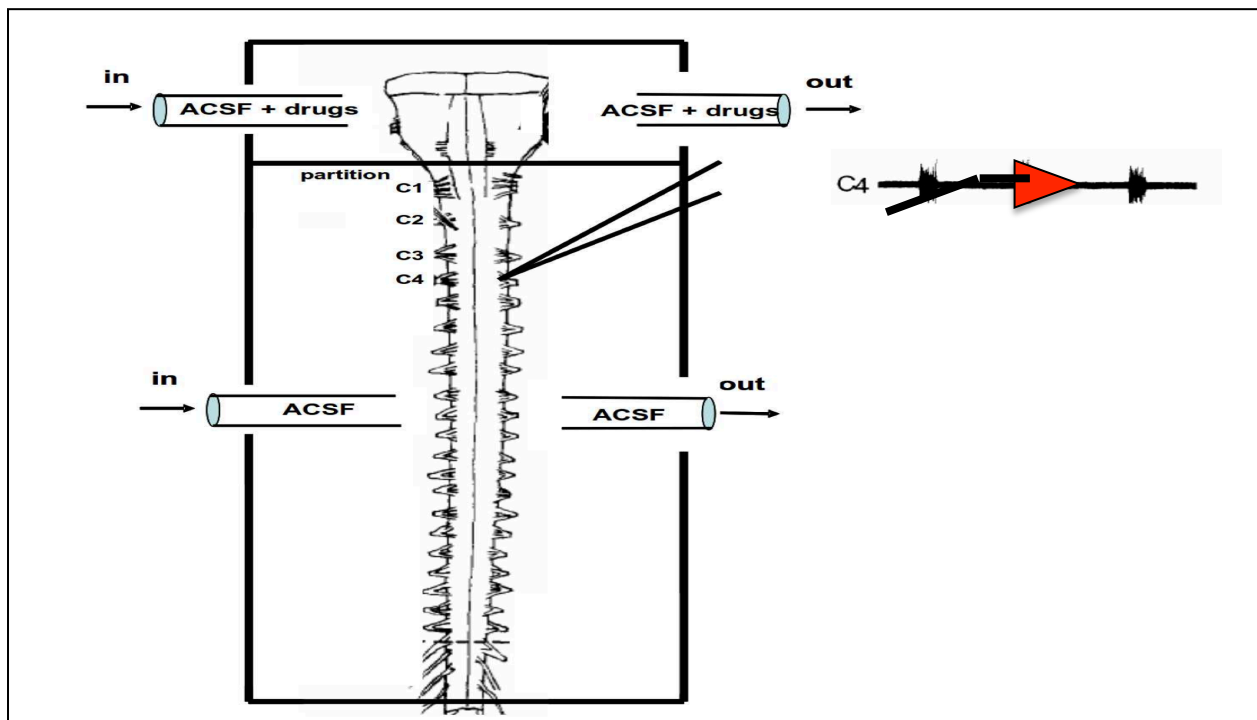


Fig. 1. Brainstem-spinal cord preparation in split-bath configuration

Experimental Protocol

The split-bath configuration allowed drugs to be administered to either the rostral chamber, only perfusing the brainstem, or the caudal chamber, only perfusing the spinal cord, without contacting the rest of the preparation. The divider was lined with petroleum jelly, in order to create a waterproof barrier between the two chambers, and held the preparation at the

base of the brainstem. The same experimental protocol was followed for both the rostral and caudal experiments, with the exception of which chamber was receiving the drugs, and which chamber was receiving aCSF. A glass pipet containing an electrode was filled with aCSF solution, so that the bottom of the electrode was covered, and was attached to a ventral nerve root from spinal segments C1-C4. A rhythm was observed in order to establish the health of the preparation, and then a baseline measurement was recorded for ten minutes during which the preparation was receiving a steady flow of oxygenated aCSF solution. After baseline, an oxygenated 5HT alpha-7 antagonist solution was administered for fifteen minutes to either the rostral or caudal chamber. Then a mixture of 5HT alpha-7 antagonist and 5HT solution, still oxygenated, was administered to the same chamber. This was followed by a twenty minute washout where both chambers were receiving a steady flow of oxygenated aCSF solution, similar to baseline conditions.

Statistical Analysis

To test the data statistically, we used 2-factor ANOVA, with treatment (DNE vs control) and time of drug application the main factors. When the ANOVA revealed significant differences, posthoc tests were done using the Bonferroni method. In all cases, $P < 0.05$ was the threshold for statistical significance.

Results

Rostral Experiment

Adding serotonin in the presence of the 5HT alpha-7 antagonist to the rostral compartment resulted in a decrease in both amplitude and frequency on both control and DNE groups. Graphical representation of amplitude of bursts and frequency of bursts for this experiment can be found on figures 2D and 3D, respectively. Figures 2A and 3A show response

to just serotonin application. Figures 2B-2C and 3B-3C are the result of previous studies where antagonists to receptor types other than α -7 were used. Figures A-C were included in order to provide a point of comparison for D figures. These results indicate that there is a significant difference in both amplitude and frequency between the control group and the DNE group. For both amplitude and frequency, the response to serotonin administration was reduced, i.e. the decrease associated with serotonin application in the control group was much smaller in the DNE group.

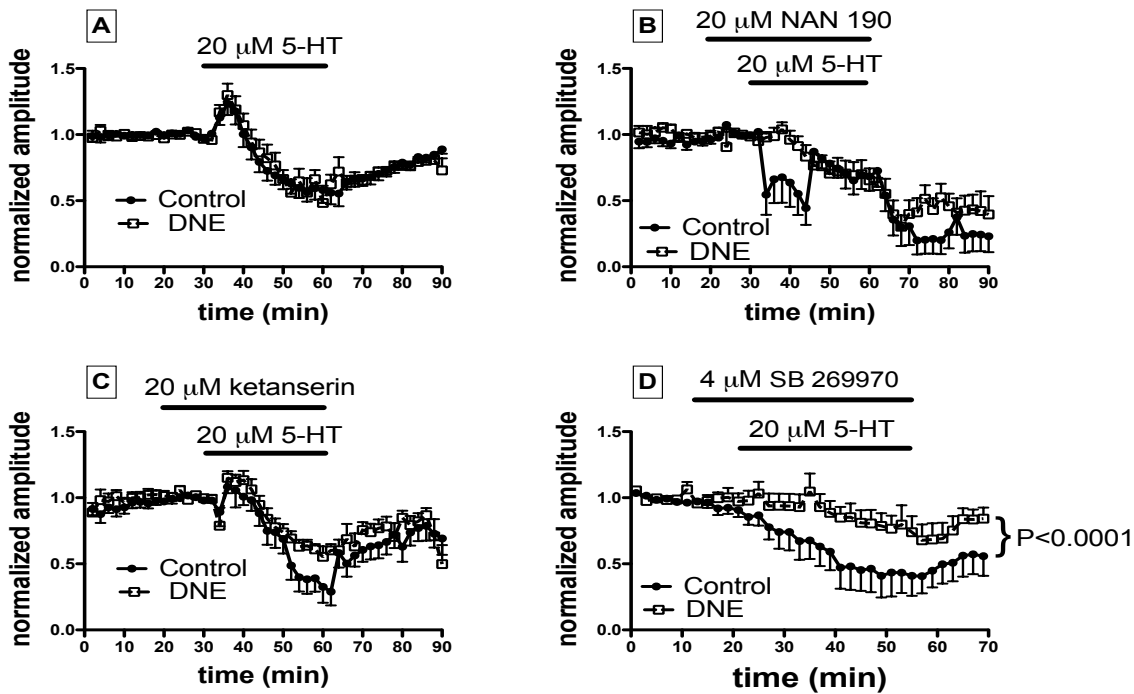


Fig. 2. *A.* Following a transient increase, frequency steadily declined in response to 5HT in controls. In DNE animals, the rise in frequency was enhanced and the onset of decline delayed. *B.* The 5HT_{1A} antagonist NAN-190 blocked the transient increase in both groups. Interestingly, washout was associated with a further drop in frequency in both groups. *C, D.* Antagonists of the 5HT_{2A} and 5HT₇ receptors abolished the transient increase in frequency in both groups, and

also attenuated the late decline in frequency in the DNE animals

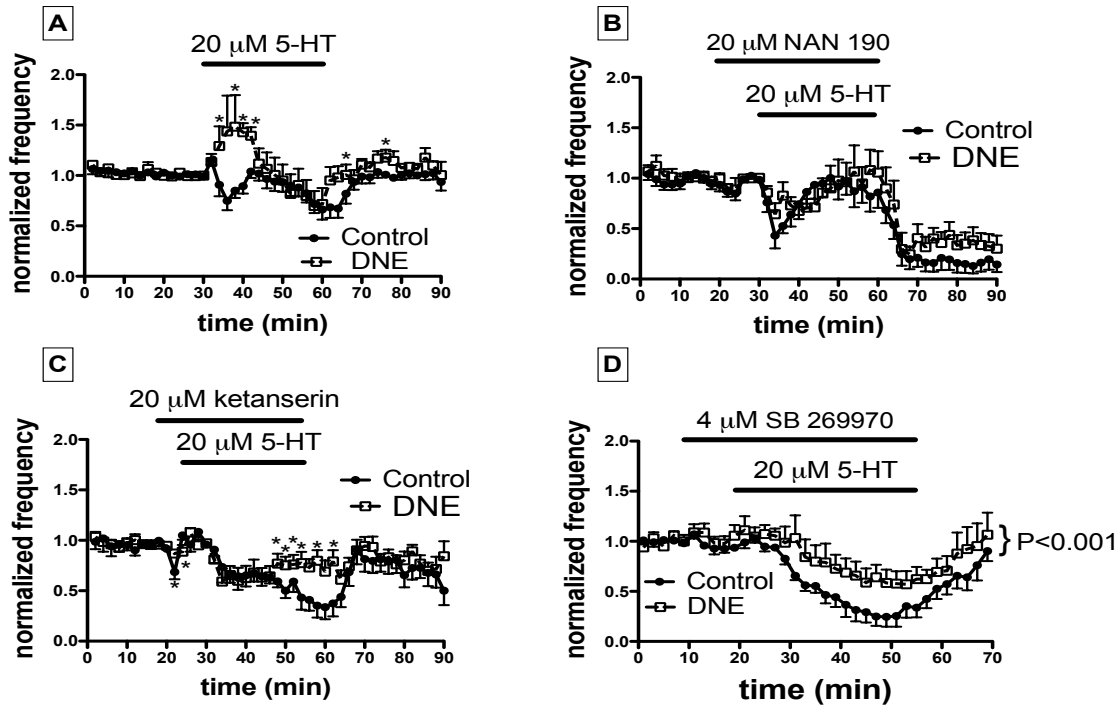


Fig. 3. *A.* Following a transient increase, C4VR burst amplitude steadily declined in response to 5HT in both treatment groups. *B.* Blocking 5HT1A receptors with NAN-190 abolished the transient increase in both groups, and appears to inhibit amplitude markedly in controls. As with frequency, washout was associated with a further drop in amplitude in both groups. *C.* Blocking 5HT1A receptors slightly attenuated the increase in amplitude in both groups. *D.* Antagonists of 5HT7 receptors abolished the transient increase in amplitude in both groups, and attenuated the subsequent decline in the DNE animals.

Caudal Experiment

Adding serotonin in the presence of the alpha-7 antagonist to the caudal compartment resulted in increase in tonic activity in both control and DNE groups. The control group increase more prominently than the DNE group, but the difference between the two groups was only significant at $P=0.013$. The results for the caudal experiment are graphically depicted in figure

4D. As in the rostral experimental figures, figures 4A show response to just serotonin application. Figure 4B-4C are the result of previous studies where antagonists to receptor types other than α -7 were used. Figures A-C were included in order to provide a point of comparison for figure D.

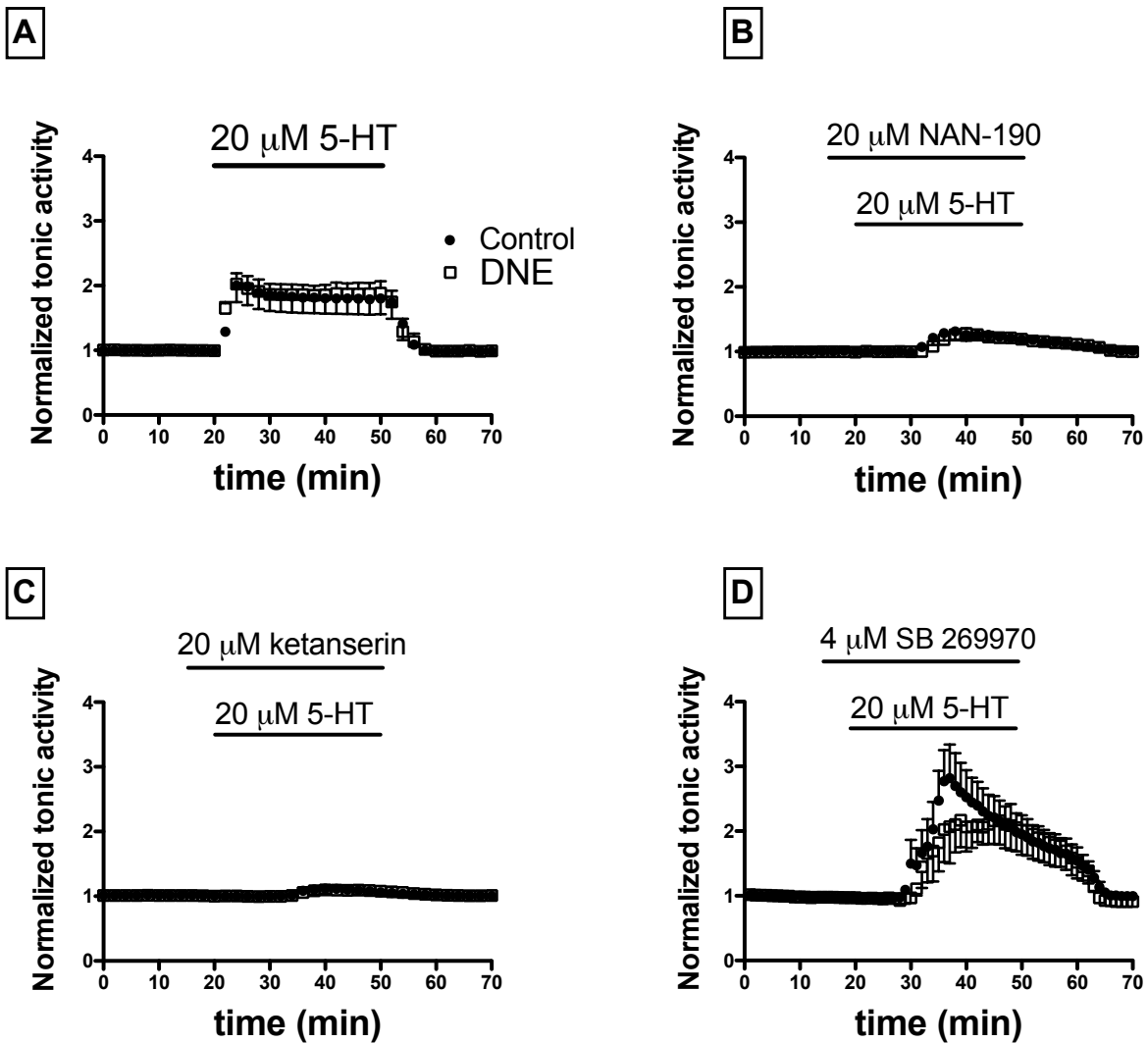


Fig. 4. A. Following application of serotonin, tonic activity increased and held at increased level until serotonin application ceased. B. Blocking 5HT1A receptors with NAN-190 greatly diminished the increase seen with solely serotonin in both groups. C. Blocking 5HT1A receptors

diminished the increase seen with solely serotonin in both groups even more than in figure B. *D*. Antagonists of 5HT-alpha 7 receptors caused a larger increase than just application of serotonin in control groups, while DNE groups increase to a similar level as seen with just serotonin application.

Discussion

Rostral Experiment

When looking at serotonin application alone, see figure 2A, both groups experience an immediate increase in amplitude above baseline, followed by a steady decline in amplitude that falls below baseline. As amplitude decreases, the slope of the line also decreases, approaching a plateau just before washout. Once washout is initiated, the amplitude begins to increase, approaching baseline. In the presence of serotonin plus 5HT alpha-7 receptor antagonist, both the control group and the DNE group lose the initial increase in amplitude that is seen with just serotonin application, see figure 2D. In the control group, the decrease in amplitude in the presence of the antagonist follows a similar pattern as is seen in the presence of just serotonin. However, in the DNE group, the decrease in amplitude is both delayed and much less drastic.

In the presence of solely serotonin, the control group demonstrates a sharp increase in frequency; followed by a sharp decrease in frequency, below baseline, within the first ten minutes of serotonin exposure, see figure 3A. This is followed by a return to baseline, which is in turn followed by a much more gradual decrease in frequency until the beginning of washout. In the DNE group, the initial sharp decrease below baseline is lost. Instead, the frequency in the DNE group increases more gradually and then returns to baseline within the first ten minutes of serotonin application, again see figure 3A. After those first ten minutes, the DNE group follows a similar pattern as the control group, following a much more gradual pattern of decrease in

frequency until the beginning of washout. In the presence of serotonin plus 5HT alpha-7 receptor antagonist, both groups lose the initial increase in frequency, see figure 3D. Instead, there is just a gradual decrease in frequency that continues until the beginning of washout. This decrease in frequency is more pronounced in the control group; where as the response in the DNE group appears to be reduced.

When comparing the control group and the DNE group, the data demonstrates that DNE reduces the inhibitory action of neurons in the brainstem in response to serotonin. The increase in the difference between these two groups in the presence of 5HT alpha-7 receptor antagonist indicates that the 5HT alpha-7 receptor plays a role in the inhibition of both burst amplitude and frequency in response to serotonin. This lack of inhibition indicates that DNE animals will have increased ventilatory output when exposed to serotonin than control animals. While having an increased ventilatory output may appear to be a positive affect, especially in the context of lack of oxygen in the blood, this will actually have the opposite affect on an animal that is presented with a breathing challenge during sleep. For example, if a blanket or pillow was covering an infant's nose, they will begin to breath in their own exhaled carbon dioxide. As a result, an increase in the amplitude and frequency of that breath will result in their blood carbon dioxide levels rising faster than a control infant's would, causing asphyxiation at a faster rate.

Caudal Experiment

The application of only serotonin to the spinal cord of the preparation, via the caudal chamber, results in a drastic increase in tonic activity in both control and DNE groups, see figure 4A. This increased level of tonic activity is then held constant above baseline for the duration of serotonin application, decreasing back to baseline once washout has begun. Figures 4B and 4C illustrate that this affect can be completely removed by blocking other 5HT receptors. When

administering serotonin plus 5HT alpha-7 receptor antagonist, the control group has an initial peak in tonic activity, followed by a gradual decrease in tonic activity until washout is begun, which causes a return to baseline. In the DNE group, however, that initial increase matches the increase seen with solely serotonin application and the animal's response follows the same pattern until washout. Tonic activity is an indicator of the excitability of the motor neurons within the spinal cord. Application of serotonin causes an increase in excitability, which is held constant until the serotonin is removed. The 5HT alpha-7 receptor appears to have an inhibitory affect on the initial spike in excitability in the control group, see figure 4D. However, the DNE group has lost this initial spike in activity, even when the 5HT alpha-7 receptor is blocked.

Conclusion

As demonstrated by the rostral experiments, 5HT alpha-7 receptor contributes to the inhibitory response of neurons in the brainstem to serotonin. Serotonin application has an inhibitory affect on both burst amplitude and frequency. This inhibitory response is reduced by prenatal exposure to nicotine, resulting in an increase in carbon dioxide concentration in the blood in the context of a breathing challenge. Serotonin causes an increase in excitability when administered to the motor neurons within the spinal cord via the caudal chamber. The 5HT alpha-7 receptor appears to inhibit an initial spike in excitability in the control group. The DNE group appears to have lost this initial spike, regardless of whether or not the 5HT alpha-7 receptor is blocked.

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