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Lesions of the paraventricular thalamic nucleus attenuates prepulse inhibition of the acoustic startle reflex

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HIGHLIGHTS

- The lesion of mPVT disrupts prepulse inhibition response.
- mPVT lesion did not affect locomotor activity.
- These results and the known connections of PVT might make it an important center in modulatory PPI network.
ABSTRACT

The paraventricular thalamic nucleus (PVT) is a midline nucleus with strong connections to cortical and subcortical brain regions such as the prefrontal cortex, amygdala, nucleus accumbens (NAc) and hippocampus and receives strong projections from brain stem nuclei. Prepulse inhibition (PPI) is mediated and modulated by complex cortical and subcortical networks that are yet to be fully identified in detail. Here, we suggest that the PVT may be an important brain region for the modulation of PPI. In our study, the PVT of rats was electrolytically lesioned. Two weeks after the surgery, the PPI response of the animals was monitored and recorded using measurements of acoustic startle reflex. Our results show that disruption of the PVT dramatically attenuated PPI at prepulse intensities of 74, 78 and 86 dB compared to that in the sham lesion group. Thus, we suggest that the PVT may be an important part of the PPI network in the rat brain.

Keywords: Paraventricular thalamic nucleus (PVT); Prepulse inhibition (PPI); Lesion; Schizophrenia
1. INTRODUCTION

The paraventricular thalamic nucleus (PVT) is one of the midline thalamic nuclei that lies on the anterior-posterior axis of the dorsal medial thalamus with densely packed glutamatergic neurons [1,2]. The major projections to the PVT arrive from the medial prefrontal cortex (mPFC) [3]; the hypothalamic nuclei, especially the suprachiasmatic nucleus, lateral hypothalamus and arcuate nucleus [3,5]; and the brainstem nuclei, such as the raphe nuclei, locus coeruleus, ventral tegmental area, pedunculopontine tegmental nucleus (PPTg), periaqueductal grey (PAG) [1,5,11-14] and superior colliculus (SC) [15]. In turn, glutamatergic PVT neurons innervate several subcortical structures, such as the basolateral (BLA) and central amygdala (CeA), together with bed nucleus stria terminalis [2,6,7]; nucleus accumbens (NAc) [8-10] and cortical structures such as the mPFC [5,16,17]. These diverse connections with subcortical and cortical structures suggest a possible modulatory role in several cognitive functions, including attention and arousal [2].

The major neurotransmitters that innervate the PVT are dopamine, serotonin, norepinephrine, orexin, corticotropin-releasing hormone and endogenous opioids [5,18-23]. Orexin is particularly interesting among these as the PVT receives the densest orexinergic input from the lateral hypothalamus among all thalamic nuclei in rodents and primates [20,24-27], and the orexinergic system is suggested to be linked to schizophrenia-related neural networks in several reports [28-30].

The connections of the PVT also make it a powerful candidate for the sensorimotor gating modulation network. Disruption in PPI is an endophenotype in patients with schizophrenia [31,32], and this response can be measured in a variety of other animals, including primates, rodents and even zebra fish [33-38]. On the other hand, there are limited reports describing the PPI networks in mammals [39-43]. Although the role of the thalamus in sensorimotor gating, and therefore in schizophrenia, has been known for a long time, thalamic structures are disregarded in the models of the PPI network. Even though the generation of the startle reflex is mainly governed by the brainstem nuclei, the mediation and modulation of the PPI response involve both subcortical and cortical structures [43]. The major subcortical players are suggested to be the PPTg, PAG and cuneiform nuclei (CuN) for the mediation of PPI. Subcortical regions such as the VTA, NAc, and BLA play key roles in the attentional and conditional modulation of PPI together with the cortical regions such as the mPFC [43]. The fact that the PVT is well-connected with the centers of the PPI network might suggest that this nucleus is a candidate modulatory region of the PPI response. Therefore, our focus in this study was to reveal the possibility of PVT involvement in PPI networks by conducting a lesion study.

2. METHODS
The experiment was carried out on 14 healthy adult male Wistar albino rats weighing 350-400 g at the time of the surgery. Animals were housed at the NPARC animal facility in cages of 5 animals with a 12/12 light-dark cycle, controlled temperature (20-24°C) and humidity (40-70%), and access to food and water ad libitum. The Local Animal Research Ethics Committee approved the study (date: 08.05.2015, number: 2015-05).

Prior to the start of experiments, all animals were handled for 5 min by the experimenter on 3 consecutive days. Each animal was caged individually post-op until the surgical site was healed and was then returned to the initial cage.

2.1. Stereotaxic surgery and lesions

Lesions of PVT were performed on a stereotaxic frame adapted for rats (Laboratory Standard Stereotaxic Equipment, World Precision Instruments, FL, USA) after anaesthesia with a 3:1 ketamine/xylazine combination. Electrolytic lesions of the PVT were performed using bipolar stainless-steel electrodes that were lowered into the brain perpendicularly through a large enough hole in the skull at the midline. The coordinates were determined using histological analysis of preliminary surgeries on rats with the same weight. Coordinates were 2.1 mm posterior from bregma (AP -2.1 mm) and 5.4 mm ventral to the dura (DV -5.4 mm). The complete electrolytic lesion of the PVT was achieved using anodal constant direct current (1.1 mA, 10 s). The sham lesion animals were exposed to the same procedure except that no electrical current was passed through the electrode [44]. The incision was sutured, and the site of surgery was cleaned with povidone-iodine. The rats were allowed two weeks of full recovery before continuing with the PPI test.

2.2. Prepulse inhibition

Sensorimotor gating responses were measured using four Acoustic Startle Reflex System chambers (SR-LAB, San Diego Instruments, San Diego, CA, USA). Each chamber consists of a plexiglass cylinder (8.8 cm in diameter, 25 cm in length) adjustable for different sizes and a plastic platform on which the cylinder was mounted. Chambers were placed inside an illuminated and ventilated box. The startle responses were transduced by a piezoelectric accelerometer, which is digitized, rectified and recorded as 100 1-ms recordings at the onset of each startle stimulus. An average of 100 readings was used as the dependent measure. Background white noise and acoustic pulse/prepulse stimuli were generated by a loudspeaker that was mounted 24 cm above the cylinder and controlled by the SR-LAB software. The system was periodically calibrated for speaker performance and stabilometer sensitivity. The detailed PPI task protocol is given in Supplementary Figure 1.

After the three-day handling procedure, all animals were habituated in the startle chambers on the fourth day for 15 min without background noise or pulses. The basal PPI responses were recorded on the fifth day with the protocol previously described by Özçetin et al. [38] The animals were grouped
such that low, medium and high average PPI responses were represented equally in each group. The lesions were performed following the basal PPI measurements. The experimental PPI measurements were performed two weeks after the surgery with the same protocol.

At the end of each recording, the percent decrease in the startle response to each prepulse intensity (PPI %) was estimated as \[ \%PPI = \left(1 - \frac{\rho_+}{\rho_-}\right) \times 100\ \text{%,} \] where \( \rho_+ \) is startle amplitude with prepulse and \( \rho_- \) is startle amplitude without prepulse [35].

2.3. Locomotor activity

Locomotor activity tests were carried out in nine sound-insulated chambers with individual air conditioning and lighting. The chambers contained a 40 cm x 40 cm x 40 cm black plexiglass floor. The activity was monitored and recorded with CCD cameras mounted on the ceiling of each chamber. The recorded activity was then digitized, numbered and analysed with the video tracking device and software (Noldus, Ethovision v3.1, Netherlands). The parameters (total travel distance and duration, velocity, mobility duration and frequency, immobility duration and frequency) were analysed only for the first 5 min [45]. Data were expressed as group averages and standard errors.

2.4. Histology

After the behavioural tests, the animals were sacrificed and the brains were fixed in 10% PFA at +4°C. Coronal slices (100-μm thick) were obtained using a Vibroslice (Campden Instruments) in phosphate-buffered saline at room temperature. Whole-brain coronal slices that included the PVT were stained with Nissl staining. Lesion size was estimated using these slices and animals with a lesion that was partial or that was extending to neighboring regions, i.e. mediodorsal thalamus, were left outside the analysis.

2.5. Statistics

Basal PPI measurements (prior to surgery) were analysed with one-way ANOVA for each prepulse intensity (74 dB, 78 dB and 86 dB) and average prepulse percentage to show that lower and higher basal PPI values were distributed evenly between control and lesion groups.

For the PPI measurements after the surgery, the significant differences between the pulse intensities and groups were primarily assessed by repeated measures ANOVA followed by post hoc tests. This step was required to identify any factor-group correlation, if existed.

The results from the experimental group (electrolytic lesion) and control group (sham lesion) were compared amongst themselves for each prepulse intensity (74 dB, 78 dB and 86 dB) and average prepulse percentage using one-way ANOVA (\( \alpha=0.05 \) in all cases, if not mentioned otherwise). The
post-surgery PPI % was also compared to pre-surgery (basal) PPI% for each prepulse intensity (74 dB, 78 dB and 86 dB) using Student’s t-test for paired samples (α=0.05 in all cases). Locomotor activity results from the experimental and control groups were also compared using a one-way ANOVA. Plots were prepared in MatlabR2016a (The MathWorks Inc., Natick, MA, USA), and the error bars represent standard errors.

3. RESULTS

The animals were distributed into experimental and control groups depending on their basal average PPI% levels. The basal PPI% values for all three prepulse intensities (Figure 1a) and average PPI% (Figure 1b) were statistically similar in both groups after distribution. This analysis confirmed that the animals with lower and higher basal PPI performances were distributed evenly in the sham and lesion groups prior to the surgery, and therefore, the post-surgery PPI performances can be directly correlated with the extent of the lesion.

Electrolytic lesions of the PVT were validated using Nissl staining on 100-µm coronal slices. The size of the lesion was measured for each animal on the Nissl-stained slices. The extent of these lesions was between AP -2.0 to -2.8 mm and DV -5.0 to -5.5 mm (Figure 2), covering the PVT. The borders of the lesions did not differ above 0.2 mm AP and 0.1 mm DV among the lesion group. The selected lesions disrupted PVT in its entirety, however, with minimal or no extension to the neighboring regions. One rat, with a larger lesion extending to mediodorsal thalamus, and one rat with a partial lesion that did not cover enough space in PVT, were excluded from the lesion group. In addition, one rat from the experimental group and one rat from the control group were excluded due to a suspicion of deafness following the surgery.

The repeated measures analysis of the PPI results revealed that there was no significant interaction between surgery and prepulse intensities. Therefore, the PPI% was collapsed across prepulse levels and analysed by one-way ANOVA. Animals with successful PVT lesions (n=5) displayed significant attenuation of PPI% for prepulse intensities at 74 dB (p = 0.033), 78 dB (p = 0.039) and 86 dB (p = 0.046) compared to that of the sham lesion animals (n=5) (Figure 3a). Average PPI% (Figure 3b) was also significantly attenuated after PVT lesion (p = 0.036).

In addition, the post-surgery PPI % was compared to pre-surgery (basal) PPI% using Student’s t-test. The comparison revealed that, only in the sham group, there is a significant increase in PPI % in response to 74 dB (p= 0.0257) and 78 dB (p= 0.027) prepulse intensities and the increase for 86 dB was not significant (p= 0.075). This result points to a possible increase in startle sensitivity in rats after surgical operation.

One week after the PPI test, all animals were subjected to an open field locomotor activity test to monitor their mobility in the first 5 min of their entry. There was no significant difference between
electrolytic lesion and sham pairs in the total distance travelled, total movement duration and immobility duration (p > 0.1; Figure 4). In addition, the velocity of movement, the duration of mobility and the frequencies of mobility and immobility were analysed and no significant difference between sham and lesion groups was found for these parameters (data not shown). The locomotor activity measurements further confirmed that the decrease in PPI response in the lesion group was not due to a decrease in mobility of the animal.

4. DISCUSSION

Our study clearly showed that the structural integrity of the PVT is crucial for the proper PPI response. The electrolytic lesions were successfully limited with PVT, with very little to no damage in the neighbouring regions. Therefore, we associate the disrupted PPI response directly with the loss of the PVT.

The network that produces the PPI response is yet to be fully revealed; however, several studies have shown a three-layered network that governs the response for the PPI of the acoustic startle reflex: (1) the startle reflex network – a group of brain stem nuclei including the ventrolateral tegmental nucleus, caudal pontine reticular nucleus and cochlear nuclei, (2) the PPI mediation network – including the PPTg, CuN, dorsolateral PAG, inferior and superior colliculus, and (3) the PPI modulation network – including the VTA, NAc, amygdala, rostral ventral pallidum, dorsal hippocampus, habenula, mPFC, mediodorsal thalamus and substantia nigra [40-43,46].

Our contribution adds the PVT as a possible central modulator, connecting the PPI modulation network with the PPI mediation network. The PVT receives information from the PPTg [12,13], PAG [11], CuN [12] and SC [15] in the PPI mediation network. This information is connected to the inputs from and the outputs to regions such as the mPFC [3,5,16,17], NAc (core and shell) [8,9,10,16], BLA and CeA [3,5,6] in the PPI modulation network. Therefore, the connections of the PVT place it midway between the regions involved in PPI mediation and modulation, most likely as a center that relays information from the PPI mediation centers in a feedback loop, associates the information arriving from the cortical centers of the PPI modulation network and sends feedback information back to the cortical and subcortical PPI modulation centers. It is interesting to note that, even though its connections suggest a more “relay center”-like role for the PVT, our results show that the lesion of the region critically disrupts the PPI response. Therefore, we suggest that the modulatory role of the PVT feedback system is crucial for the proper functioning of the PPI network. It should, however, be noted that the electrolytic lesion also disrupts the fibers en passage as well as the neuronal cell bodies of PVT and therefore, it is possible that our results could reflect this effect of the lesions. Further studies with more delicate lesion methods, e.g. excitotoxic lesions, should follow to fully identify the role of PVT.
One interesting observation from the present study is that there are some increases in the PPI % of sham group for all three prepulse intensities. Statistically, there was a significant increase for only 74 dB and 78 dB prepulse intensities after the surgery. Both sham and lesion groups were raised and tested under the same conditions both pre- and post-surgery. Furthermore, they received the same surgical operation up to the point of lowering the electrode. Since the environment was strictly controlled and aging is not generally established to increase PPI %, it can be concluded that the surgical attempt on PVT, even just as lowering the electrode, is somehow increasing the startle sensitivity. The significant effect for only lower prepulse intensities further point out the sensitivity increase. In the lesion group, this sensitivity might be masked due to the lesion itself.

Disrupted PPI performance is an endophenotype for neurodevelopmental disorders such as schizophrenia [31,32]. Therefore, it is widely used as a symptomatic validation of partial animal models of these disorders [46]. The mPFC-thalamus connection is known to be involved in schizophrenia pathophysiology [47]. The PVT is a very strong candidate for being the thalamic center in this corticothalamic connection through its dense reciprocal communication with the mPFC [48]. PVT is also suggested to play a modulatory role for the dopaminergic signalling systems [29,49, 50], that are also linked to the modulation of cognitive symptoms in schizophrenia [49,51,52]. Although the claim that the PVT is a part of the schizophrenia network might currently be an overreach, our results and the current literature suggests a possible connection that should be further investigated in detail.

5. Conclusion

In this study, the structural integrity of the PVT is shown to be crucial for the proper PPI response in rats. We suggest a possible explanation for this result by highlighting the connections of the PVT with the other brain regions involved in the production and modulation of the PPI response. Further investigations about the role of this nucleus are definitely required, and we suggest that a detailed look into the function of the PVT from this perspective will reveal its potential as a central modulatory target in the networks associated with neurodevelopmental disorders.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Disclosure

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

Contributions:
PÖ designed the study. PÖ, TG, and AÖ performed the lesion studies. PÖ and FDKY gathered behavioural data. PÖ, FDKY, and İTU analysed and interpreted the results. PÖ, FDKY, and İTU wrote the paper.

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Figure Legends

**Figure 1: Basal prepulse inhibition percentages.** a) Basal PPI% of the sham lesion group (n=5) and PVT lesion group (n=5) for the prepulse intensities at 74 dB, 78 dB and 86 dB. There was no significant difference between the groups, indicating an even distribution of low, medium and high basal PPI% between groups. b) Similar to the prepulse intensity responses, the average basal PPI% also had no significant difference between the sham lesion and PVT lesion groups.

**Figure 2: Location and dimensions of electrolytic lesions.** A) Example lesion images from lesion group animals around AP -2.1 mm, which was the site of current injection. Even though the AP length of the lesions slightly differed among the animals, the middle section of PVT was consistently lesioned and the lesion size was also similar in all animals. Each image is scaled to 700 µm width. B) The extent of the electrolytic lesions of the PVT. The grey circles in the figure displays the average size of the lesion from all animals in respective AP coordinates. There was negligible lateral extension to mediodorsal thalamus in only one lesion.
Figure 3: Prepulse inhibition percentages after surgery. a) PPI% for prepulse intensities at 74 dB, 78 dB and 86 dB were all significantly attenuated in the PVT lesion group (n=5) compared to the sham lesion group (n=5) (* p < 0.05). b) Similar to the prepulse intensity responses, the average PPI% was also attenuated in the PVT lesion group.

Figure 4: Locomotor activity results after surgery. The total distance travelled (a), the total movement duration (b) and the immobility duration (c) in the first 5 min. are displayed in the figure. The error bars represent standard error. There was no significant difference in the locomotor activity performances between sham and lesion group.