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Neuropeptide Y Affects Thalamic Reticular Nucleus Neuronal Firing and Network Synchronisation Associated with Suppression of Spike-Wave Discharges

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Running title- NPY: mechanisms of seizure suppression

Key Points:

- Neuropeptide Y increases interictal neuronal firing in thalamic reticular nucleus (NRT) after intracerebroventricular (ICV) and local juxtacellular injection
- Neuropeptide Y decreases ictal waveform correlations between cortex and NRT
- Neuropeptide Y ICV injection increases stimulation threshold in S2 somatosensory cortical area for the induction of absence-like seizures

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ABSTRACT

Objectives: Neuropeptide Y (NPY) potently suppresses spike-wave discharges (SWDs) in a genetic rat model of absence epilepsy (GAERS), but the underlying neurophysiological mechanisms are not clear. We therefore sought to determine the in-vivo effects of NPY on neuronal firing in the cortico-thalamo-cortical network activity, known to play a critical role in the generation of SWDs in these rats.

Methods: NPY was administered intracerebroventricularly (ICV) or in separate experiments locally on the neurons of caudal thalamic reticular nucleus (NRT) by use of juxtacellular iontophoresis in triple barrel electrodes in male GAERS aged 12-15 weeks, *in vivo* under neuroleptic anaesthesia. Drug infusions and EEG monitoring were performed simultaneously with juxtacellular single neuronal recordings. NPY effects on electrically-induced SWD induction threshold were also measured.

Results: NPY administration ICV led to a decrease in the total length of SWDs in EEG recordings. Both ICV administration and iontophoresis of NPY on NRT neurons led to an increase in interictal neuronal firing of NRT neurons. During ictal periods, ICV NPY administration reduced the number of thalamic action potentials per SWDs, as well as reduced waveform correlations between field potentials within the NRT and the cortical EEG. NPY administration ICV did not significantly alter the firing patterns of relay thalamic neurons interictally and cortical neurons during ictal and interictal periods. Additionally, SWD induction threshold in S2 region of the cortex was significantly increased after NPY administration.

Significance: Our results show alterations in cortico-thalamo-cortical local and network properties following ICV administration of NPY, suggesting mechanisms of SWD suppression in GAERS. Cellular and network alteration of NRT activity, resulting from a direct action of NPY, may be a contributor to this effect.

Introduction

Absence seizures are one of the most common seizure types in patients with genetic generalized epilepsy (GGE), and are associated with a number of syndromes including childhood absence epilepsy, juvenile absence epilepsy and juvenile myoclonic epilepsy.¹ Currently available treatments for absence epilepsies often do not provide complete seizure control in a significant number of patients, and can have significant adverse effects.^{2, 3} Endogenous neuropeptide-related mechanisms are potential novel targets for new classes of anti-epileptic drugs. Of particular interest has been neuropeptide Y (NPY). NPY is widely and abundantly expressed in the mammalian CNS⁴ and is a member of the pancreatic polypeptide family with three subtypes, Y1, Y2 and Y5. NPY is co-localized with several other neurotransmitters and is a critical inhibitory regulator of neuronal excitability.^{5, 6} A growing body of literature suggests that NPY plays an anti-epileptic role in acquired focal epilepsy in patients^{7, 8} as well as in animal models of acquired epilepsies.^{9, 10}

The seizures of genetic generalised epilepsies, particularly absence seizures, involve pathological oscillations of the cortico-thalamo-cortical circuit. Genetic absence epilepsy rats from Strasbourg (GAERS) provide a well-characterised rat model of absence epilepsy that display spontaneous absence-like seizures with generalised SWDs generated by pathological cortico-thalamo-cortical oscillations. These oscillations possibly originate from abnormal rhythmic high frequency activity within the S2 somatosensory cortex.¹¹ We have previously shown seizure suppressant effects of NPY in this animal model of absence epilepsy.^{4, 12-14} Intracerebro-ventricular (ICV) injections of

NPY significantly suppressed SWDs in this model.¹² Moreover, administration of agonists for specific receptor subtypes Y1, Y2 and Y5 showed that activation of Y2 receptor subtype was most important for suppression of SWDs.⁴

Despite these reports, the underlying mechanisms for NPY mediated SWD suppression in GAERS remain obscure. Therefore, we aimed to investigate the effects of NPY administration on neuronal firing and pathological network activity in the cortico-thalamo-cortical brain regions, which are critical structures in the generation of SWDs. We performed juxtacellular neuronal recordings from neurons of these networks before and after ICV administration of NPY in GAERS rats. In addition, we performed focal administration of NPY on caudal NRT neurons that were being recorded by juxtacellular iontophoresis. Glass microelectrodes (15-30mΩ) filled with neurobiotin were used for extracellular recordings and allowed subsequent neuronal identification after iontophoretic labelling. We also evaluated the effects of NPY administration on the threshold of SWDs induced by a 2-s train of 7-Hz electrical stimulation in the S2 somatosensory cortex. We show a significant role of the NRT in the mechanism of NPY mediated SWD suppression in the GAERS model of absence seizures.

Materials and Methods

Experiments were conducted on 12-15 week old male GAERS. Animals were housed in standard small animal lab cage and kept in a temperature controlled animal room maintained at 22-24°C with a 12hr light/dark cycle with lights on between 7am-7pm. All electrophysiological recordings were performed during the light phase. All experiments conducted in this study were approved by the Melbourne Health Animal Ethics Committee. All procedures were conducted in accordance with the requirements of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and followed the National Health and Medical Research Council Guidelines to promote the wellbeing of animals used for scientific purposes.

Anaesthesia and surgery

All surgical procedures were performed under general anaesthesia (ketamine/xylazine in 3:1 ratio; 0.1ml /100gm, KX) and electrophysiological recordings were performed under neurolept anaesthesia (*d*-tubocurarine (2mg/1ml of water), fentanyl (0.2ml), haloperidol (0.2ml) with glucose), which provides deep anaesthesia and paralysis but provides an electrophysiologically pseudo-awake state, allowing the spontaneous occurrences of SWDs.

The rats were anaesthetised with an intraperitoneal injection of KX. Once adequately anaesthetised, tracheostomy and penile vein catheterization (0.8x0.4mm polyethylene tubing, Microtube Extrusions) were performed. The tracheal tube was then connected to a ventilator with ventilation at 60 breaths/minute $PH_2O = 8-12$. The rats were then mounted on stereotaxic frame (David Kopf instruments, Tujunga, CA, USA). Heart rate, body temperature and oxygen were continuously monitored and maintained at physiological limits ($PO_2 > 90\%$, Heart rate 250-400 beats/minute, temperature $37^\circ C$) at all times during the experiment. Two mono-polar needle electrodes were implanted subcutaneously on each side of the thorax to measure electrocardiogram (ECG). An active EEG electrode was epidurally implanted on the parietal cortex for cortical electroencephalogram (ECoG). For ICV injections an ICV cannula was implanted in the left hemisphere (A/P: 1mm, M/L: -1.5 mm and -3.5mm deep relative to dura). Placement of cannula was confirmed by withdrawing cerebrospinal fluid through the ICV injecting line. The electrodes and the ICV cannula were then secured in position using dental acrylate and a dummy line was then inserted into the ICV guide cannula to avoid blockage of the guide. Neurolept anaesthesia was initiated before the end of general anaesthesia, and was administered continuously with micro injection pump (KDS 230, KD scientific) at 0.5ml/hr.

Electrophysiology:

Paired Glass Electrode recordings:

Two craniotomies and duratomies were performed on the right hemisphere of the skull (A/P: 2.6 mm, M/L: 4.8 with -12.5° and A/P: 3.6 mm, M/L: 3.6 mm) for paired electrode recordings. To avoid desiccation, these openings were covered with surgical sponge soaked in saline. Borosilicate glass capillaries of 1mm OD x 0.58mm ID, (Harvard Apparatus) were pulled to tip diameter of ≈ 1 micron (15-25 $m\Omega$ in saline). These glass micropipettes were back filled with 1.5% neurobiotin (50mg of neurobiotin in 3.3ml of 1M potassium acetate) and were lowered into the desired brain region using a stepping microdrive (Omnidrive, Neurostar, Germany) with $2\mu m$ steps and at relatively low speed of 0.04mm/sec. After finding stable target neurons, paired juxtacellular single neuronal recordings were performed simultaneously with cortical EEG. Stable juxtacellular single neuronal firing was recorded with the injection of Saline and NPY at different time points. Baseline neuronal recording was performed for the first 15 mins followed by ICV administration of $2\mu l$ saline into left lateral ventricle of the brain. Post-saline juxtacellular recordings were performed for 15 minutes. This was followed by ICV administration of $2\mu l$ of 1.5nmol NPY followed by 30 minutes recording. The recorded cell was then labelled with neurobiotin by applying positive currents pulses (0.5–8 nA) through the micropipette tip (200 ms on/200 ms off for around 10 minutes). Transcardial

perfusion was then performed with 4% paraformaldehyde (PFA) and the brain was removed for histological study (Fig. 1A-C).

Focal injections of NPY:

For the focal injection study, a single craniotomy and duratomy was performed at the right hemisphere of the brain (A/P: 3.6 mm, M/L: 3.6 mm). A double barrel borosilicate glass electrode was pulled to a diameter of ≈ 2 micron (15-30 M Ω). One barrel was back filled with 1.5% neurobiotin and other barrel was filled with saline or NPY (0.5nM) for control or treatment investigations respectively. The electrode was then slowly lowered to caudal NRT to locate a stable firing neuron. 15 mins of baseline recordings was then followed by 15 min of saline or NPY injections, post saline/NPY recordings were performed with cortical EEG simultaneously. The injections were performed using a microiontophoresis technique (Union-40 iontophoresis pump, Kation Scientific). We used 200nA of ejection current (+ve) for 10 minutes to deposit NPY (0.5nM) focally on the recording neuron and a retaining current of 40 nA was applied at all times during the recordings. Recorded cells were again labelled iontophoretically for later histological identification.

SWD induction by cortical stimulation:

For these experiments, single craniotomy and duratomy was performed (A/P 0.2mm, M/L 4.1). A borosilicate glass capillary was pulled to form a sharp tip, which was then broken gently to achieve a resistance of 2-5 m Ω . It was then backfilled with 1.5% neurobiotin solution in 1 M potassium acetate and positioned in the somatosensory (S2) cortex with the stepping microdrive. A baseline recording was performed for 15 mins to assure stability. Baseline SWD inducing threshold was assessed by applying an external electrical stimulation (7Hz). Currents of 1-10milliAmps were applied by increasing 1milliamp each step until the SWDs were induced, Saline/NPY was then administered ICV and SWD inducing threshold was assessed 15min, 1hour and 2 hours post intervention. At the end of the experiment the whole region was labelled iontophoretically for further histological identification.

Histology:

After the experiment, the animals had transcardial perfusion with 4% paraformaldehyde (PFA) and the brain was removed and sectioned to visualise the labelled cell/region. Histology was performed on free-floating sections. The whole brain was sectioned into 100 μ m sections with a vibrotome

(Microm HM 650 V) and sections were placed in a 24 well plate. These sections were then washed 3 times with 0.1 M phosphate buffer solution (PBS). After washing, 400µl of ABC (Avidin/Biotin complex, ABC kit, Vector laboratories) solution was added to each well and left overnight on rotating stage. Next day, these brain slices were again washed 3 times again with 0.1M PBS and stained with DAB solution (buffer 15%, DAB 2.4%, Hydrogen peroxide 1.5%, nickel chloride 5%, DAB peroxidase substrate kit, Vector laboratories). The stained brain slices were then washed with distilled water and mounted on glass slides. The sections were then observed under bright field inverted microscope (Olympus BX 51) under 20 x magnification to identify labelled cells (Fig. 1B-C).

Data acquisition and analysis:

Data acquisition and basic analysis of the neuronal activity was performed using Clampex software (PCLamp 9, Molecular Devices). Single cell activity was recorded with a Neurodata dual channel recording amplifier (Cygnus technology Inc). Signals were passed through a programmable signal conditioner (Axon instruments, CyberAmp 380) and digitized at 20000 KHz with an Axon Instrument 1440 analog to digital converter.

The three standardized periods – baseline, post-saline injection and post-NPY injection (Fig.1D) were subdivided into ictal (seizing period), interictal (non-seizing period) and pre-ictal (transition period from non-seizing period to seizing period, 5 seconds before the start of SWDs) periods. Different firing parameters were defined to quantify the neural firing patterns in ictal and interictal periods. Five parameters were used to evaluate neuronal firing patterns: (1) Mean action potential (AP) firing frequency, (2) Percentage of AP's in burst, (3) Mean number of AP's per burst, (4) Maximum number of APs per burst, (5) Intraburst frequency as previously described.^{15, 16}

Parameters for characterization of cell firing patterns in ictal periods were (1) the number of APs firing per SWD, (2) the percentage of action potentials firing in burst during SWDs. We also evaluated EEG data to determine the effect of NPY on SWDs including total length, mean duration of SWDs and cycle frequency.

Autocorrelation analysis:

The rhythmicity of the spontaneous neuronal firing was assessed by using autocorrelation analysis method developed by Kaneoke.¹⁷ In this method, an autocorrelogram is constructed using interspike intervals. A Lomb periodogram is used to detect rhythmicity in an autocorrelogram. This method allows detection of significance of rhythmicity in spontaneous neuronal firing and also enables the

detection of multiple frequencies in an autocorrelogram.¹⁸ These measures were assessed over a frequency range from 0 to 50Hz.

Waveform Correlation

Waveform correlation (Spike 2 software, version 5.2.1, CED, UK) was used to measure correlation between waveforms recorded from two different regions in the brain.¹⁹ The signals of interest were imported into Spike 2 software and processed to produce a cross correlogram. The peak of the cross correlogram close to zero is calculated and the result of waveform correlation is scaled from -1 (correlated but inverted), through 0 (uncorrelated), to +1 (full correlation). Waveform correlations were assessed between cortical EEG and field potentials recorded from NRT region during the ictal, interictal and pre-ictal phases and were compared before and after the NPY administration.

Statistical analysis:

Statistical analysis was performed using the Graph Pad (Prism Software). Non-parametric repeated measure ANOVA was performed to analyse the statistical difference between the treatments. Relevant comparative t-test was performed to assess significance between two individual treatments. Fisher's exact test was used to evaluate changes in the proportion of rhythmic cells following treatment. All data are expressed as mean $\pm$ standard mean unless specified otherwise in the figure legend. P-values less than 0.05 are considered significant.

Results:

NPY suppressed SWDs in rats under neuralept anesthesia

In previous studies, we have shown a strong effect of NPY administration on SWD suppression in freely moving GAERS.^{4, 12} Consistent with this, in this current study, we showed ICV administration of NPY in GAERS under neurolept anaesthesia produced a strong suppression in SWDs. We observed a significant decrease in the total time spent of SWDs after ICV NPY administration without a change in mean SWD duration or cycle frequency, when compared to the saline treatment as well as the baseline period (Fig. 2A-C).

NPY administration affects interictal Neuronal firing patterns in the NRT

Juxtacellular recordings performed from caudal NRT neurons displayed a significant increase in mean (**tonic**) firing frequency interictally (122% increase from baseline: n=12 cells from 8 rats) after administration of ICV NPY when compared to the period of baseline recording (Fig. 3A-B). The

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increase in firing frequency after NPY injection did not reach statistical significance compared with the periods recorded after saline injection. This prompted us to perform an independent experiment, to compare firing frequency following two subsequent 15 minutes periods of recordings with the baseline periods. No significant effects of first or second injection of saline were observed compared to the baseline periods (Figure 3C). No alteration in ictal firing was observed in NRT neurons following NPY administration (Figure 3D).

For ventrobasal (VB) thalamus, although there were no changes observed during interictal period, the **tonic** action potentials per SWD during ictal period were significantly reduced after NPY administration (n=8 cells from 7 rats), when compared to the baseline **recordings (Fig. 3E-G)**. However, no differences were observed in other interictal or ictal neuronal firing parameters **including burst firing**, evaluated for NRT or neurons recorded in the VB thalamus or cortical regions (data not shown).

NPY does not affect rhythmicity of NRT neurons

We next sought to evaluate whether NPY administration affects local neuronal network properties of recorded NRT cells. For this we evaluated number of total recorded cells that displayed rhythmic firing. Rhythmicity of firing was quantified by performing a spectral analysis of the autocorrelogram to generate a Lomb periodogram. The proportion of cells firing rhythmically was identified and compared before and after NPY administration during the three different phases including ictal, pre-ictal, interictal periods. No significant change was observed in proportion of rhythmic cells before and after the ICV administration of NPY during ictal period (Fig. 4A-C) or during pre and interictal periods (not shown).

NPY administration alters network synchronisation between cortex and NRT:

To assess the effect of NPY on correlation between large interconnected neuronal networks, we performed cross correlation between cortical EEG and local field potentials from NRT in the cortico-thalamo-cortical circuit. As local field potentials arise from the summation of neuronal activity of a number of cells in a particular region, cross correlations of these field potentials is likely to provide a measure of network functional connectivity. Cross-correlations were performed using the Spike-2 software between the pair of electrodes from -1 to +1s, where observation of a peak near time displacement zero suggested a correlated and synchronous signal.¹⁹ Mean peak value were compared; a significant decrease in the correlation of field potentials was found between cortex and NRT with ICV NPY administration during SWD activity (n=12 cells, 8 rats, p=0.01; Fig. 4D-F). However, no differences were observed while analysing the effects during the preictal or interictal periods (data not shown).

Focal administration of NPY on recorded caudal NRT alters their firing patterns:

We next investigated whether the changes seen in NRT firing observed with ICV injection represented a direct effect or a secondary network effect. This was done by directly applying NPY to the NRT cells by iontophoresis while simultaneously recording from them using a multi-barreled electrode. As with ICV administration, local application of NPY led to a significant increase in the mean firing frequency of the recorded NRT neurons during the interictal periods (n=9 cells from 9 rats, $p=0.003$; Fig. 5A). Separate experiments were also performed, one with the iontophoresis electrode filled with NPY and the second experiment type with saline iontophored. The percentage difference between saline or NPY injection when compared to baseline was calculated. A significantly greater increase in the percentage change of mean firing frequency was observed following NPY infusions when compared to saline electrophoresis (n=9 rats, $p=0.0002$; Fig. 5B)

Effects of NPY on SWD induction threshold:

We have previously shown that external electrical stimulation in cortical regions including motor cortex, insular cortex and somatosensory S1 and S2 regions, successfully triggers SWD events in GAERS.¹¹ In particular, the S2 region required the lowest intensity stimulation to induce SWDs, consistent with reports that the S2 region may be critical in generation of SWDs¹³. We therefore sought to evaluate here whether ICV administration of NPY would alter the threshold of this type of induced activity from cortical regions. The SWD induction threshold in the somatosensory cortex was assessed before and after the ICV administration of NPY. SWD induction threshold before NPY administration was 0.91 ± 0.51 mA (n= 8 rats) that was significantly increased to 2.91 ± 0.76 mA 15 minutes after ICV NPY infusion (n=8, $p=0.02$; Fig. 6).

Discussion:

In this study, we show that ICV NPY administration suppressed absence-like SWDs in GAERS under neuroleptic anesthesia, similar to what has previously been reported in freely moving rats. This effect was associated with increased interictal neuronal firing within the caudal NRT and this increased firing was replicated by focal iontophoretic application of NPY to single cells – indicating a direct effect of NPY on these cells. NPY administration also led to reduced waveform correlation between the NRT and cortical region during ictal periods as well as a reduced number of action potential of thalamic VB neurons with respect to each SWD. ICV NPY also increased the threshold to SWD induction by electrical stimulation of S2 somatosensory cortex.

Pathological oscillations within reciprocal connections of cortico-thalamo-cortical circuits, involving somatosensory cortex, VB and NRT, are responsible for the initiation and maintenance of absence seizures in both humans and rats.²⁰ The NRT is regarded as a critical structure in the cortico-thalamo-cortical circuit as the rhythmic hypersynchronous oscillations that characterize absence seizures are dependent on this network.²¹⁻²³ The NRT projects recurrent inhibitory synapses to VB thalamus²⁴⁻²⁶ and receives glutamatergic inputs from cortico-thalamic and thalamo-cortical neurons.^{27, 28} The NRT is composed of GABAergic neurons and regulates the rhythmic oscillatory cortico-thalamo-cortical activity via GABA-mediated hyperpolarization of VB thalamus neurons, thereby de-inactivating low threshold T-type calcium channels, resulting in high frequency action potential bursts.^{22, 29, 30}

The neurophysiological and neuronal network effects underlying NPY-mediated suppression of SWDs in GAERS rats^{4, 12} have largely been unexplored. In the current study, we have demonstrated that both ICV and local injections of NPY on recorded neurons from caudal NRT *in vivo* result in an increased firing frequency of these neurons interictally. NPY mediated alteration of NRT firing did not include any change in percentage of action potential firing in bursts. Bacci et al.³¹ showed several effects on evoked synaptic currents in neocortical tissue, that would be expected to enhance interneuron activity and reduced excitability of pyramidal neurons. Although Bacci et al.'s observations were on synaptic currents rather than firing properties and performed in non-epileptic *in vitro* brain slice, the mechanisms described are compatible with our results observed in the NRT region. The increase in firing frequency of caudal NRT neurons without any changes in pathological bursts may contribute to SWD suppression by phasically increasing GABA activity in the thalamus. Such a change may inhibit pathological oscillations within this circuit and therefore reduce SWD generation. This is supported by our finding that during ictal periods VB neurons fire lower number of action potential during a SWD after ICV NPY administration. Previous studies have shown differential effects of blocking NRT neurons on suppression of SWDs³², based on whether whole NRT or only the caudal region are lesioned.³³ These findings suggest distinct roles of caudal NRT neurons recorded in our study that project to the thalamic relay neurons to that of the rostral NRT that projects to the anterior thalamic nucleus.³⁴ It remains to be seen whether the rostral NRT is affected differently to the ICV or focal application of NPY. Overall our findings suggest an important role of the caudal NRT in the mechanisms of NPY mediated SWD suppression in GAERS. Studies reporting absence seizure inhibition using antiepileptic treatments have also shown an increased expression of NPY in the NRT region^{14, 35}, consistent with our hypothesis.

Absence seizures involve the synchronous oscillatory firing of neurones within the cortico-thalamo-cortical circuit. Therefore, it was also of interest to investigate whether the ICV administration of NPY resulted in disruption of the correlation between the firing of neurons in different regions in this

network. To examine this, we performed autocorrelation within NRT neurons as well as waveform correlations between NRT and somatosensory cortical EEG. Interestingly a decrease in waveform correlation of field potentials between cortex and NRT was found during SWDs after NPY administration. This reduced synchrony may play a role in the reduced overall SWD duration we observed with NPY. Future studies should explore this further, using more sophisticated analysis network methods, including non-linear approaches.

Previous results from our laboratory have shown that SWDs can be activated in GAERS animals by 7 Hz electrical stimulation of insular and somatosensory (S1 and S2) cortex.¹¹ The lowest threshold for this was found in the S2 region, which has been identified as the likely region for seizure initiation.¹¹ Using the same technique, we have shown in this study that NPY increased the threshold stimulation needed to elicit SWD's in the somatosensory cortex of GAERS. This may be a result of enhanced inhibitory drive from NRT on thalamus from increased interictal NRT spiking, perhaps through enhanced GABA inhibitory input. Additionally, it is possible that there may be a desynchronising effect induced by enhanced tonic NRT firing preventing sustained hyperpolarisation of relay neurons that may produce rebound excitation and burst firing.^{29, 30} However, it is also likely that other factors related to the frontal cortical region are also involved. This includes the observation of Van Raay et al¹³ that NPY has maximal seizure suppression effect when injected into the S2 region, as well as Bacci et al's³¹ observation of enhanced inhibition in interneurons and reduced pyramidal cell excitatory drive in frontal cortex brain slices. Moreover, findings from Avanzini et al, (1992)³² also suggested that blocking conductance from thalamic neurons did not alter SWDs and from Van Raay¹³, showing that focal injections of NPY directly in the thalamic neurons aggravated SWDs, suggest a complex interplay of structures within this network.

The expression of NPY is reported to be specific to the GABAergic neurons in the NRT region within the lateral thalamic regions.^{35, 36} However, NPY receptor subtypes Y1, Y2, and Y5 have all been demonstrated to be present in cortico-thalamo-cortical circuit.^{37, 38} NPY specific receptor subtypes Y1 and Y5 are primarily located in postsynaptic neuronal locations and may act directly to inhibit their activity by activation of G-protein-activated inwardly rectifying potassium (GIRK) channels.³⁹ Y2 receptors are located at presynaptic positions on nerve terminals of neurons and act to inhibit GABA and glutamate release via inhibition of voltage-gated calcium channels.^{39, 40} We have previously shown that the suppressant effects of NPY on SWD activity is primarily mediated via the Y2 receptors in freely moving GAERS.⁴ This is consistent with finding in current study as an increased activation of Y2 receptors within the NRT may reduce postsynaptic inhibition and enhance excitation to promote tonic firing. Alternatively, SWD inhibiting actions of NPY may be via its postsynaptic receptors present within the NRT and thalamic relay cells.³⁶ However, a direct action of NPY on thalamic relay cells is unlikely since it has been previously shown that NPY administration

in the relay thalamic cells can further aggravate absence seizures in GAERS.¹³ Further experiments with NPY receptor subtype Y1, Y2 and Y5 agonists and antagonists in vivo will be needed to clarify receptor subtypes involved.

In conclusion, the results of this study show a significant effect of NPY administration on the firing patterns of the NRT in the GAERS model of absence epilepsy. This suggests that the NRT plays a critical mechanistic role in the SWD suppressant effects of NPY in this model. Future studies, using multi-electrode approaches may allow a larger number of neurons to be sampled within the cortico-thalamo-cortical circuitry to better understand the network connectivity properties affected by NPY administration. Moreover, intracellular and whole cell patch recordings are necessary to shed further light on synaptic mechanisms involved in the NPY effects.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figure legends:

Figure 1: Illustrations of labelled neurons in recorded regions and experimental time line for paired juxtacellular recordings.

Figure 2: Effect of NPY on pathological SWDs in EEG. (A) Representative trace of EEG recording from a rat under neuralept anesthesia before and after ICV administration of NPY. Graphs quantifying (B) the total length of SWDs and (C) mean duration of SWDs and (D) cycle frequency of SWDs. ** $p < 0.01$ and *** $p < 0.001$; $n = 11$ rats.

Figure 3: Effect of NPY on neuronal firing patterns. (A) Representative trace of a juxtacellular recording, showing NRT neuronal firing before and after ICV administration of NPY during the interictal period. Graphs quantifying (B) the **tonic** firing frequency of the NRT neurons during interictal recordings comparing the periods of saline and NPY administration with baseline, ** $p < 0.01$; $n = 12$ cells from 8 rats. (C) Control experiments comparing effects of two saline injections 15 minutes apart with the baseline recording periods on the firing frequency of NRT neurons ($n = 6$ rats). (D) Number of action potentials firing for NRT neuron per SWD during the ictal phase ($n = 12$ from 8 rats). (E) interictal firing frequency of ventrobasal thalamic neuron and (F-G) number of action potential firing in thalamic neurons per SWD during ictal periods ($n = 8$ cells from 7 rats).

Figure 4: Effect of NPY on rhythmicity and neuronal network connections of NRT cells. An illustrative trace from a juxtacellular recording showing rhythmic and non-rhythmic neuronal action potential firing. (B) An illustration of a spectrum of an autocorrelogram to calculate the rhythmicity of neuronal firing based on time stamps of action potentials is shown from one animal. (C) Graph quantifying the proportion of rhythmic and non-rhythmic neurons during the ictal period, comparing between the baseline, saline and NPY administration ($n = 12$ cells from 8 rats). (D) Representative trace showing paired recordings of field potential from NRT neurons with EEG before and after NPY administration during ictal periods. (E) A waveform cross-corrologram from one animal generated by using Spike 2 program showing a weak positive peak at time 0 between the field potentials in NRT and the corresponding cortical EEG. (C) Graph comparing the correlation values between the baseline period and after the NPY administration * $p < 0.05$; $n = 12$.

Figure 5: Effect of Focal NPY injection on neuronal firing in NRT. (A) Graphs quantifying the firing frequency of a NRT cell before and after NPY injection as well as (B) percentage change from baseline firing after saline or NPY injection. ** $p < 0.01$, *** $p < 0.001$; $n = 9$ cells from 9 rats.

Figure 6: Effect of NPY on SWD induction threshold. (A) An exemplary picture of a stimulated SWD similar to the spontaneous SWDs observed in absence seizures in GAERS induced by a cortical stimulation in the S2 somatosensory cortical region. (B) Graph illustrates seizure threshold in the S2 region comparing between the ICV saline and at 15 min, 1 hr and 2 hr after NPY injections, * $p < 0.05$; $n = 8$ rats.

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