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RESEARCH ARTICLE

Synchronous neuronal interactions in rat hypothalamic culture: a novel model for the study of network dynamics in metabolic disorders

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Abstract

Synchronous neural activity is a feature of normal brain function, and altered synchronization is observed in several neurological diseases. Dysfunction in hypothalamic pathways leads to obesity, suggesting that hypothalamic neural synchrony is critical for energy homeostasis. The lateral hypothalamic orexin neurons are extensively interconnected with other brain structures and are important for energy balance. Earlier studies show that rats with higher orexin sensitivity are obesity resistant. Similarly, topiramate, an anti-epileptic drug, has been shown to reduce weight in humans. Since orexin enhances neuronal excitation, we hypothesized that obesity-resistant rats with higher orexin sensitivity may exhibit enhanced hypothalamic synchronization. We further hypothesized that anti-obesity agents such as orexin and topiramate will enhance hypothalamic synchronization. To test this, we examined neural synchronicity in primary embryonic hypothalamic cell cultures, obtained from embryonic day 18 (E18) obesity-susceptible Sprague–Dawley (SD) and obesity-resistant rats. Hypothalamic tissue was cultured in multielectrode array (MEA), and recordings were performed twice weekly, from 4th to 32nd day in vitro (DIV). Next, we tested the effects of orexin and topiramate application on neural synchronicity of hypothalamic cultures obtained from SD rat embryos. Signals were analyzed for synchronization using cross correlation. Our results showed that (1) obesity-resistant hypothalamus exhibits significantly higher synchronization compared to obesity-sensitive hypothalamus; and (2) orexin and topiramate enhance hypothalamic synchronization. These results support that enhanced orexin sensitivity is associated with greater neural synchronization, and that anti-obesity treatments enhance network synchronization, thus constrain variability in hypothalamic output signals, to extrahypothalamic structures involved in energy homeostasis.

Keywords Hypothalamus · Obesity · Orexin · Topiramate · Synchronization

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Introduction

Regulation of energy balance depends upon integration of internal factors (nutritional status, leptin, insulin) and external factors (nutritional cues) (Baskin et al. 1999; Berthoud 2004; Schwartz 2006; Cornier et al. 2007). The process by which brain integrates these signals to maintain healthy body weight is under intense investigation. It is known that there are key neuroendocrine signals to specialized neurons of the hypothalamus reflecting body energy status, which in turn participate in energy balance (Williams 2012). Dysfunction of hypothalamus can result in chronic positive energy balance and weight gain (Kotz et al. 2008; Tregellas et al. 2011; Knutson 2012; Williams 2012).

The lateral hypothalamus (LH) was historically identified as a hunger and wakefulness-regulating center (Moruzzi and Magoun 1949; Delgado and Anand 1953) which contains neurons directly sensitive to changes in plasma glucose levels (Anand et al. 1964). Orexin neurons, which found in this hypothalamic region, play a pivotal role in energy sensing, sleep/wake regulation, feeding behavior and energy balance (de Lecea et al. 1998; Liu et al. 2011; Belanger-Willoughby et al. 2016). We have shown that resistance to weight gain in obesity-resistant (OR) rats is associated with increased LH orexin gene expression, orexin sensitivity, physical activity and enhanced sleep quality (Teske et al. 2006; Kotz et al. 2008; Mavanji et al. 2010). We and others have shown reduced weight gain following orexin treatment (Novak and Levine 2009; Perez-Leighton et al. 2012). In addition, we showed that sleep disturbances alter feeding and energy homeostasis (Mavanji et al. 2010, 2013), which could be due to altered LH orexin function. Orexin is known to enhance neural synchronization, excitability and synaptic plasticity in the locus coeruleus (LC) (van den Pol et al. 2002; Selbach et al. 2004; Yamanaka et al. 2010). Understanding the relationship between orexin-mediated obesity resistance and sleep requires knowledge of the temporal dynamics of neural activity in the hypothalamus, and how orexin alters the network dynamics in this neural structure.

Topiramate is an anticonvulsant agent (Shank et al. 1994), which induces weight and fat loss in humans (Reife et al. 2000; Bray et al. 2003; Astrup et al. 2004; Wilding et al. 2004; Tonstad et al. 2005; Rosenstock et al. 2007; Tremblay et al. 2007). Based on these findings, topiramate was explored as a potential treatment for the general case of obesity and binge eating disorder (De Bernardi et al. 2005; Guerdjikova et al. 2005; Vorsanger et al. 2016) and now forms part of the FDA-approved obesity combination medication known as Qsymia. Our unpublished data and other studies show reduced food intake and body weight after topiramate treatment in rats (Picard et al. 2000; York et al. 2000; Richard et al. 2002; Husum et al. 2003; Abo-Elmatty and Zaitone 2011; Simko et al. 2014).

Levin and colleagues showed that, when fed a high-energy diet, more than half of out-bred Sprague–Dawley rats developed diet-induced obesity, while the remaining rats were resistant to obesity. We refer here to these rats as standard Sprague–Dawley (SD) and obesity-resistant (OR) rats. The SD rats share many characteristics of human obesity and follow a polygenic mode of inheritance (Levin et al. 2000). Hypothalamic synaptic organization shows qualitative and quantitative differences between SD and OR rats, indicating differences in hypothalamic function between obese-prone and -resistant phenotypes (Williams 2012). Thus, evaluating potential differences in hypothalamic network function between obese and lean phenotypes may yield valuable clues about neurobiological mechanisms of obesity.

Multielectrode array (MEA) recording technique, which allows in vitro measurement of neuronal interactions, can be used to further our understanding of the neurobiology of body weight regulation. The MEA technique informs us about how much neurons work together in small or large ensembles to form dynamic neural networks. To date, neural network studies identifying the dynamics of complex networks of brain regions involved in energy balance are sparse. The MEA technique has permitted real-time study of neuronal activity and spatial and temporal interactions between neurons in developing neural networks (Potter and DeMarse 2001; Marom and Shahaf 2002; Wagenaar et al. 2006). Earlier studies have shown the importance of pairwise correlations in describing the dynamics of a network (Schneidman et al. 2006)]. Cross correlation analysis provides an estimate of the synchronicity status of the network on a millisecond-by-millisecond basis.

To understand the dynamics of neural interactions within the hypothalamus, we measured network synchronization in in vitro hypothalamic neural networks obtained from SD and OR rat embryos. We hypothesized that OR rats with higher LH orexin sensitivity exhibit greater neural synchronization in the hypothalamus. We also hypothesized that orexin, and body weight reducing topiramate, would enhance synchronicity in embryonic hypothalamic neuron cultures. Here, we report that the OR hypothalamus exhibited highly significantly increased synchronized network activity. In addition, we showed that orexin and topiramate enhance synchronicity in hypothalamic cultures, potentially reducing variability in postsynaptic output from the hypothalamus to energy balance-regulatory areas, which might facilitate obesity prevention.

Methods

Ethical approval

Use of rat tissue was approved by the local Institutional Animal Care and Use Committee at the Minneapolis VA Healthcare System and University of Minnesota (IACUC ACORP# 140801). All authors understand the ethical principles under which the *Journal of Physiology* operates and the current work complies with the animal ethics checklist as outlined in the journal's editorial.

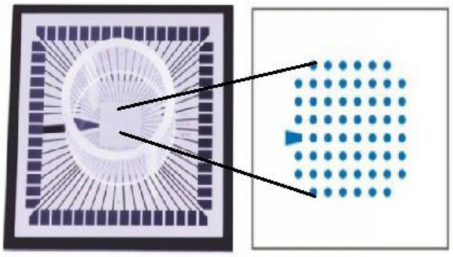

Fig. 1 A picture of MEA used in these experiments (https://www.multichannelsystems.com/) (left panel) and magnified depiction of electrode arrangement (right panel)

Hypothalamic cell culture

Timed mated pregnant Sprague–Dawley (SD) and obesity-resistant (OR) rats obtained from Charles River Laboratories (Kingston, NY, USA) were anesthetized using CO_2, and embryonic day 18 (E18) embryos were collected (Experiment 1). For experiment 2, E18 embryos were collected from pregnant SD rats. We used one pregnant rat for each of the multielectrode array (MEA) plates. We used three to four male embryos per plate and the whole hypothalamic tissue including the lateral hypothalamic area for the current studies. Brains were dissected and hypothalamic tissue was dissociated from the rest of the brain, as described elsewhere (Gao and van den Pol 2001). Cells were isolated immediately after dissociation and were plated on MEAs as described previously (Christopoulos et al. 2012). In brief, hypothalamic tissue was placed in media (Neurobasal/B27 + 0.5 mM Glutamax; Invitrogen, Carlsbad, CA) and was mechanically triturated to obtain dissociated cells. The sample that contained the dissociated cells was spun, supernatant was discarded and the pellet was re-suspended in Neurobasal media. The cells in the suspension were counted using a hemocytometer. Live cells were determined by dye exclusion. Approximately, 250,000 hypothalamic cells in 100 µl of media were pipetted and plated directly onto and around the electrode area of each sterile MEA (Fig. 1). Prior to plating, MEAs were cleaned with 70% ethanol, autoclaved, and sterilized under UV light for 2 h. Sterilized MEAs were then coated overnight with poly-D-lysine (Sigma; 50 mg/ml in water). Poly-D-lysine was then aspirated, and MEAs were rinsed once with sterile water and allowed to dry under the hood. After plating, the MEAs were covered by a Teflon cover and placed in an incubator for 1 h at 37 °C in a humidified incubator containing 5% CO_2 to allow the cells to adhere to the MEAs. After this, the media were removed and replaced with 0.9 ml of fresh, pre-incubated Neurobasal media. Fresh media were made weekly, and one-half of the media (0.45 ml) in each MEA was replaced twice a week.

Data acquisition

Each MEA (MultiChannel-Systems, Reutlingen, Germany; model MEA120-2-System) is embedded with 60 electrode sites on a flat surface surrounded by a circular wall that creates a well around the electrodes (Fig. 1). The electrodes are titanium nitride disks (30 µm diameter) arranged in a square grid with 200 µm spacing between disks. Recording of neuronal activity was performed twice weekly, beginning at 4th day in vitro (DIV) and ending at 32nd DIV, for each of the eight MEAs (4 OR and 4 SD hypothalamus; Experiment 1) plated with cells, as described earlier (Christopoulos et al. 2012). For experiment 1, independent cultures obtained from separate pregnant rats were used. As mentioned above, we used four OR and four SD independent hypothalamus cultures, with each culture consisting of hypothalami obtained from three to four embryos of the same mother. Thus, one pregnant rat contributed to one specific culture. Briefly, to record the neuronal activity, MEAs with plated cells were removed from the incubator and placed in a temperature-controlled (37 °C) amplifier assembly and recording modules located within a Faraday shielded metal box. Recording sessions began between 1100 and 1200 h. Electrical activity from each electrode was preamplified (low-pass filter 15 kHz), amplified (1.0–3.0 kHz band-pass filter) and sampled at 20 kHz using the amplification stages and data recording modules built into the MEA hardware. Signals from all 60 electrodes in an MEA were recorded for 1 min using the MultiChannelSystems software MC_Rack (Version 3.9.1). In the second experiment, we tested the effects of orexin and topiramate on hypothalamic synchronous activity. To do this, orexin-A (American Peptides) was suspended in artificial cerebrospinal fluid (aCSF) to have a 300 micromolar solution. Topiramate was suspended in aCSF to have a concentration of 100 µmol. The dosage of orexin and topiramate was based on earlier published studies (Gordey

et al. 2000; Duffy et al. 2016). Prior to recording, a third of the MEA cultures ($n=4$) were treated with topiramate (100 µmol; Janssen Pharmaceuticals, obtained through Minneapolis VA Healthcare system's pharmacy) and the other third ($n=4$) were treated with aCSF (1 µl). The remaining MEA cultures ($n=4$) were treated with orexin (1 µl of 300 micromolar orexin, to obtain a final concentration of 300 nmol in 1 ml). Recording sessions began immediately following the application of either orexin, topiramate or aCSF into the MEA.

Data preprocessing

Local field potentials (LFP) were derived from the neuronal data recorded at 20 kHz by applying a second-order bandpass Butterworth filter at 0.7–170 Hz to reject frequencies outside the LFP range (Fig. 2). The filtered LFP were then down-sampled to 1 kHz for further analysis, yielding a 60,000 time sample series from each electrode. Noisy and saturated electrodes were visually detected and excluded from further analysis.

Earlier observations with the LFP time series revealed that they are non-stationary with respect to the mean, and highly auto-correlated (Christopoulos et al. 2012). Because our aim was to assess the interaction between LFP time series obtained from each of the 60 electrodes, using cross correlation functions, it was required that individual series be rendered stationary and non-auto-correlated. Accordingly, by appropriate preprocessing, the time series were converted into white noise (prewhitening) as described earlier (Christopoulos et al. 2012). The prewhitening was achieved in two stages: model identification and model parameter estimation (Christopoulos et al. 2012). We used the Auto-Regressive Integrative Moving Average (ARIMA) model to pre-whiten the LFP time series. The ARIMA modeling was carried out using Matlab (R2010b, version 7.11.0.584, 64 bit). The basic ARIMA equations can be found in an earlier publication (Christova et al. 2011). Earlier work indicated that the ARIMA model is adequate and yields residuals (innovations) that are stationary and non-auto-correlated, and can be used for cross correlation analysis (Christopoulos et al. 2012).

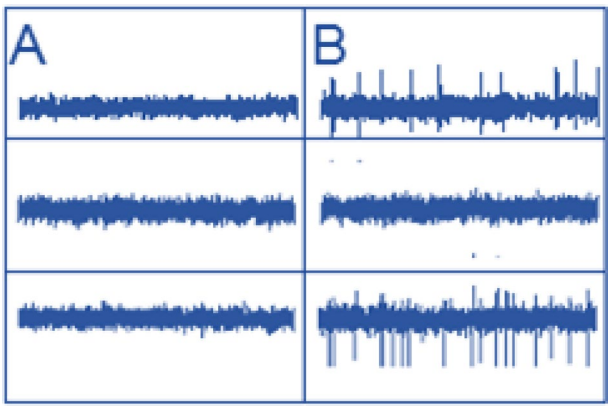

Fig. 2 Raw LFP from SD (**a**) and OR rats (**b**) obtained from three MEA channels each

Statistical analyses

We assessed the synchronicity between neuronal populations by calculating all pairwise zero-lag cross correlations between prewhitened LFP time series (ARIMA innovations, see above). For statistical analyses, we took the absolute value of CC (since its sign is not relevant) and z-transformed it (Fisher 1958) to normalize its distribution: zCC = atanh(CC). Statistical comparisons were tested using ANCOVA where group (control, obesity resistant) was a fixed factor and DIV was a covariate for the first experiment. For the second experiment, ANCOVA was performed where group/treatment (control, orexin, topiramate) was a fixed factor and DIV was a covariate. Matlab R2010b, version 7.11.0.584 (64 bit), the IMSL statistical library in FORTRAN (Compaq Visual Fortran Professional Edition, version 6.6B) and the IBM-SPSS statistical package (version 23) were used for all analyses.

Results

The aim of the first experiment was to test whether the magnitude of synchrony changes as the hypothalamic cultures from SD and OR embryos grew in vitro. Since there was no change in network activity (strength of cross correlations) across recordings days, all the recording days were pooled. We found that hypothalamic cultures from obesity-resistant embryos (experiment 1) exhibit significantly higher synchronous neural interaction compared to cultures from control embryos (Fig. 3; $F\,(1{,}102{,}288)=512.8$, $P<0.001$; F test in ANCOVA).

In the second experiment, we tested the effects of orexin, topiramate and control vehicle on the synchronous neural interaction of embryonic hypothalamic tissue obtained from control Sprague–Dawley rats. We found that both topiramate and orexin enhanced synchronous neural interaction compared to vehicle in the hypothalamic culture, indicating specificity of the effect (Fig. 4; $F\,(199{,}419)=251.8$, $P<0.001$; F test in ANCOVA). All pairwise comparisons yielded statistically significant difference following treatment. ($P<0.001$, control vs. orexin; $P<0.001$, control vs. topiramate, $P<0.001$ topiramate vs. orexin), but synchronous neural interaction was highest in the orexin-treated cultures (Fig. 4).

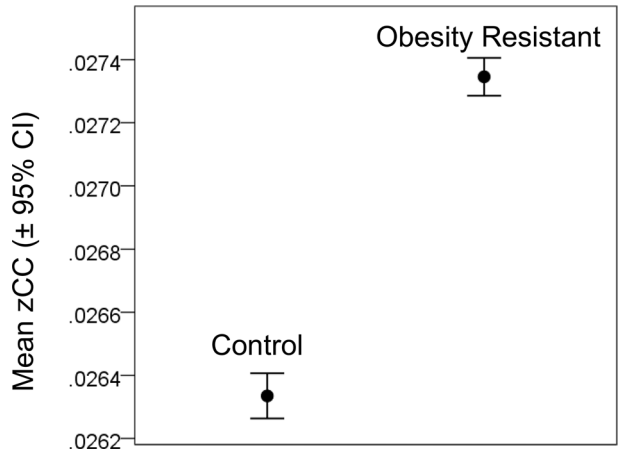

Fig. 3 Mean z-transformed zero-lag cross correlation ZCC [(±95% confidence intervals (CI)] for control and obesity-resistant MEA cultures. $n = 32$, MEA × DIV for control and obesity-resistant conditions

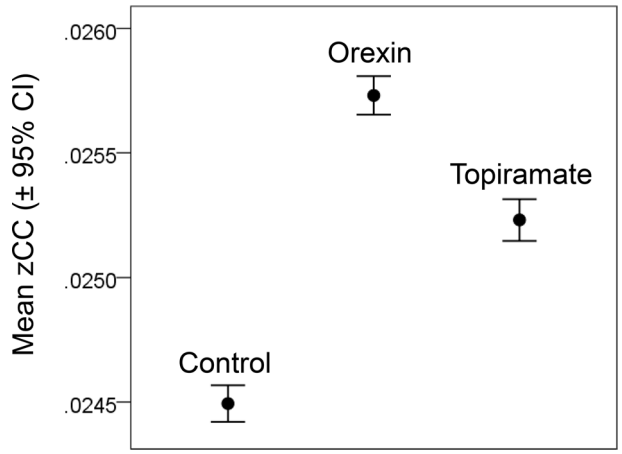

Fig. 4 Mean z-transformed zero-lag cross correlation ZCC [(±95% confidence intervals (CI)] for control, orexin and topiramate-treated hypothalamic MEA cultures. $n = 32$, MEA × DIV for control, orexin and topiramate treatments

Discussion

The current findings, for the first time, demonstrated synchronization in obesity-resistant and obesity-susceptible embryonic hypothalamic cultures. Our results showed greater synchronous neural interaction in the hypothalamus of obesity-resistant compared to obesity-susceptible rat embryos. Further, our results found higher synchronicity following application of orexin and topiramate, which have weight-reducing effects. Altogether, these findings further point to an enhanced synchronous neural interaction in the hypothalamic network as a key feature of obesity resistance.

Current results are in agreement with earlier electrophysiological studies, which demonstrated the involvement of orexin in enhancing neuronal excitability, and synchronization between neural structures (Chen et al. 2016). In one study, local field potentials were simultaneously recorded from the medial prefrontal cortex and the cerebellum of guinea pigs undergoing trace eyeblink conditioning (Chen et al. 2016). The local field potentials revealed that theta-frequency (5.0–12.0 Hz) oscillations in the medial prefrontal cortex and the cerebellum became strongly synchronized following presentation of an auditory conditioned stimulus. Further, orexin receptor-1 antagonist SB-334867 injected into the cerebellum impaired the synchronicity between medial prefrontal cortex and cerebellum, suggesting that orexin contributes to the theta band synchronization among different brain regions. In addition to theta synchronization, orexin is involved in the production of theta activity, as well as induction of sharp waves and slow-onset long-term potentiation in the hippocampus (Selbach et al. 2004; Bocian et al. 2015). In another study, application of orexin into locus coeruleus slices increased membrane depolarization, spike frequency, temporal synchrony of action potentials, and synchrony of locus coeruleus output (van den Pol et al. 2002). One of the mechanisms for temporal synchrony elicited by orexin in the locus coeruleus could be orexin-induced strong regular membrane voltage oscillations (van den Pol et al. 2002), which might be due to orexin-mediated induction of a Ca(2+)-dependent resonance in membrane potential (Ishibashi et al. 2015). At the cellular level, it is possible that orexin enhances Ca(2+) conductance, and influx into neurons, with differing temporal response profiles ranging from spiking to smooth depolarization plateaus (Kohlmeier et al. 2004; Xia et al. 2009; Peltonen et al. 2010; Putula et al. 2014). Orexin enhances overall neuronal excitability and excitatory synaptic transmission and increases the frequency of excitatory postsynaptic currents (Jeon et al. 2015). A recent study showed spike broadening and stabilization of neuronal firing rate in raphe neurons following orexin application (Ishibashi et al. 2016). Overall, enhanced calcium influx mediated neuronal excitability, higher spike frequency, spike frequency stabilization, depolarizing plateau potentials, spike broadening, slow-onset long-term potentiation, sharp waves, and synchronization of action potentials, which appear to be the mechanisms for increased synchrony after orexin application.

The mechanism by which topiramate enhances synchrony in hypothalamic culture is unknown. However, the known pharmacological mechanisms (Garnett 2000; Shank et al. 2000; Shank and Maryanoff 2008) for topiramate include: positive modulatory effect on the activity of GABA at GABAA receptors; negative modulatory effect on the activity of glutamate at kainate/alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; negative modulatory

effect on voltage-dependent sodium channels; negative effect on high voltage-activated calcium channels (McNaughton et al. 2004; Xu et al. 2012); inhibition of L-type Ca^{2+}currents (McNaughton et al. 2004). In addition, topiramate inhibits depolarizing plateau potentials and reduces epileptiform synchronization (D'Antuono et al. 2007, 2010). All these mechanisms lead to inhibition of neuronal excitation, unlike orexin. However, topiramate action might also be determined by the neural structure (with characteristic discharge properties) to which it is applied and the neurotransmitters present, to determine synchronous firing of the neurons, and the exact mechanism by which it enhances hypothalamic synchronization is yet to be determined.

Lateral hypothalamic orexin neurons are extensively interconnected with other hypothalamic areas important for sleep and energy balance, such as the arcuate nucleus, supraoptic nucleus, preoptic area, dorsomedial nucleus, tuberomammillary nucleus, posterior hypothalamus and paraventricular nucleus (Spinazzi et al. 2006; Yoshida et al. 2006; Burt et al. 2011; Williams 2012). Orexin fibers also project to cortical/limbic areas and the autonomic and motor systems of the brainstem (Espana et al. 2005), which underpins the regulation of sleep–wake behavior, energy homeostasis, as well as cognitive, reward-related, and emotion-related functions (Vittoz and Berridge 2006; Mahler et al. 2012; Yang et al. 2013). In addition, feedback mechanisms are in place to affect the excitability of the orexin neurons (Burt et al. 2011). Modulation of orexin neurons by these feedback mechanisms, and modulation of hypothalamic and extrahypothalamic neural activity by orexin may be important to the regulation of energy homeostasis. Thus, by synchronizing the hypothalamic output to extrahypothalamic structures, orexin may play an important role in regulating wakefulness, feeding, activity and energy homeostasis.

Orexins enhance synaptic connectivity and neural excitability, thus providing a molecular prerequisite for homeostatic and behavioral state-dependent control of neuronal plasticity (Yamanaka et al. 2010). On the other hand, obesity in humans and animal models cause profound changes (including inflammation) to energy balance centers of the hypothalamus, including deficient neuronal connectivity and synaptic plasticity (Williams 2012). In the current study, OR rats with higher orexin sensitivity exhibited higher synchronization in the hypothalamus. In addition, orexin and topiramate enhanced hypothalamic network synchronization. Thus, enhancing hypothalamic synchronicity might be a tool for combating obesity.

From the results, it is interesting to note the differences in neuronal excitation in embryonic hypothalamic cultures between OR and SD phenotypes, despite the obesity usually being evident until adulthood. The neurons are harvested when they are still immature, and in the developing stage; thus, caution should be taken while extrapolating these results into adult brain. However, the following evidence supports the idea that the differences in synchronicity between OR and SD hypothalamus during the developmental stage might be an indication of obesity resistance, and might be persisting into adulthood. For example, in mouse, the majority of the neuroendocrine neurons become post-mitotic between E12 and E14, and migrate to their final destinations, form discrete nuclei and start to produce specific neuropeptides (Jaworski and Perez-Martinez 2006; Dalvi et al. 2011). Most of the hypothalamic neuroendocrine regulators reach maturity around day E18 (the day tissue were harvested for the current study) (Cariboni et al. 2014). Dichotomy in neurochemical signature among OR and obesity-prone (OP) rats have been demonstrated as early as postnatal-day 0 (P0; which is 3 days after the tissue harvest day for the current study). Pharmacological studies using OP and OR rats also showed that amylin increases VMH leptin signaling in P0–P6 OP rats, and this response is also observed in adult OP rats (Dunn-Meynell et al. 2016). In addition, postnatal amylin treatment from P0 to P16 improved arcuate nucleus to paraventricular nucleus (ARC–PVN) pathway development in OP rats and caused them to gain less body weight during the amylin administration (Dunn-Meynell et al. 2016), indicating that neuronal responses are different in OR and OP rats during early development. Moreover, a dynamic difference was observed in the numbers of both proopiomelanocortin (POMC) and neuropeptide-Y (NPY) neurons in the arcuate nucleus (ARC) during the first week of postnatal life in both OP and OR neonates (Dunn-Meynell et al. 2016), again indicating that differences in neurochemical signatures among OR and OP rats emerge early during development. Supporting the idea that physiological status during embryonic development persists into adult hood, a study showed that fetal exposure to maternal undernutrition has lifelong consequences for physiological and metabolic function (Ware et al. 2015). Thus, we believe that the differences in synchrony observed in developing neural networks obtained from SD vs. OR rats might persist into adulthood in vivo resulting in the respective phenotype.

The present study has some limitations. Lack of a study addressing dose response relationship between orexin and topiramate dose, and neural synchronization is one such limitation. Future studies will be designed to assess the dose-dependent effect of orexin/topiramate on hypothalamic neural synchrony. Another limitation is that embryonic cell culture data have its own pitfalls for data interpretation in that the neurons are harvested when they are still immature, and in the developing stage; thus, the results may not be completely extrapolated into an adult brain. We are standardizing methods to culture adult primary hypothalamic neurons, and future studies will address the effects of obesity resistance, orexin and topiramate on the synchronization of adult hypothalamic

cells. In addition, the importance of the current findings in understanding neural basis of obesity is supported by earlier studies which demonstrated that the dissociated cells establish a network around 5 days in vitro and can survive up to 40 days. The cells build synaptic connections with each other during the first days of culturing, forming subnetworks and exhibiting spontaneous electrical activity. Once mature, MEA cultures exhibit strong interconnections and synchronized bursts of activity across the entire network separated by few seconds of quiescence (Kamioka et al. 1996; Christopoulos et al. 2012). Thus, it is possible that the embryonic cultures mature into stable network, similar to that observed in vivo. For example, cortical EEG obtained from neonatal cats exhibit small amplitude and low-frequency synchronous rhythmic spiking. These results suggest that the slow spontaneous rhythmic activity which is seen at early developmental stages of dissociated cortical culture is also a feature of intact intracortical networks of neonatal cats (Kamioka et al. 1996). Also, this pattern of synchronized regular bursting is observed not only in primary dissociated culture but also in slice cultures (Kamioka et al. 1996), again indicating that the dissociated culture resembles the activity patterns in vivo. Overall, the resemblance electrical activity of dissociated cells and the low-frequency spontaneous discharges observed in neonatal cortex in vivo and in slice preparations suggest the possibility of normal maturation of dissociated cells in vitro (Kamioka et al. 1996). One more limitation of the current study is that the primary cultures were heterogeneous in nature and contained a mixture of various neuronal and glial cell populations. Thus, to understand the role of synchronization fully in obesity resistance, future studies will explore the role of synchronization in specific hypothalamic neurons (anatomically and neurochemically distinct) in obesity resistance. Yet another limitation is that effects of orexin antagonists on hypothalamic synchronization is not tested here. Even though we did not study the effects of orexin antagonists on synchronization in SD and OR rats, our earlier pharmacological/chemogenetic studies on orexin and orexin antagonists indicate that orexin plays a role in obesity resistance. For example, earlier studies from our laboratory showed that orexin antagonist administered into the ventrolateral preoptic area of the hypothalamus (VLPO) (pretreatment with antagonist) blocked the increase in wakefulness and the reduction in NREM sleep elicited by orexin-A, and the OX2R antagonist reduced energy expenditure induced by orexin-A (Mavanji et al. 2015). We also showed that chemogenetic inhibition of orexin neurons reduces physical activity and energy expenditure in mice, again pointing to the anti-obesity effect of orexin (Zink et al. 2018). However, future studies will address the effect of orexin antagonists on neural synchronization in embryonic and adult hypothalamus neural cultures.

In summary, energy homeostasis requires coordinated activities among distributed hypothalamic and extrahypothalamic nuclei (Cone and Simerly 2011). One mechanism by which this can be achieved is by enhancing temporal integration of hypothalamic neural activities to induce synchronous firing, and hence synchronized output to extrahypothalamic structures. Our results indicate that orexin enhances synchronization, which might be one of the mechanisms for its anti-obesity effect. The experiment with topiramate further strengthens the idea that enhancing synchrony in the hypothalamus has therapeutic value in combating obesity. Thus, orexin and topiramate by enhancing synchronicity in the hypothalamus, might constrain variability in postsynaptic output within the hypothalamus, and from the hypothalamus to extrahypothalamic structures. The resulting temporally synchronized synaptic output from the hypothalamus should result in coordinated activities among distributed brain regions involved in energy balance, resulting in better body weight control.

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Author contributions The study was carried out at the Minneapolis VA Health Care System. VM, APG, and CMK conceived and designed the study; APG, and CMK obtained funding; VM and APG acquired and analyzed the data; VM, APG, and CMK interpreted the data; VM, APG, and CMK drafted the manuscript and critically revised it for important intellectual content. All authors approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

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