Endogenous Opioid Modulation on the Thalamo-Striatal-Cortical Circuit

Roopa Bhat, Eric Minner

Abstract

The medial thalamus, anterior cingulate cortex, and striatum are all crucial regions that provide significant contributions in reward learning as well as pain transformation. Opioids have long been used for treatment of pain, and the mechanisms through which they play on modulation of the above circuit has only recently been explored. In order to determine the concentration needed to trigger movement in this circuit, we measured the endogenous release of opioids via biochemical ligand binding assays that suggest high binding affinity of enkephalin to multiple opioid receptor subtypes. Next, we implemented whole cell recordings of pyramidal anterior cingulate cortex (ACC) neurons based on inputs from the mediodorsal thalamus (MThal) and striatal medium spiny neurons (MSN Str), injecting 1 nm and 10 nm concentrations of endogenous opioid ligand based on the results of the previous experimentation. And finally, we created a simple neural network to determine the relationship between endogenous enkephalin concentration and the circuit's role in learning activation. Our results show that the net effect of the thalamo-cortical-striatal circuit shows a duality of excitatory/inhibitory effects based on endogenous opioid release, leading to more key evidence in the vital role that opioids play in modifying pain/reward learning.

Introduction

Clinically, opioids are commonly utilized to inhibit sensory discernment and motivational dimensions of pain by acting as modulators for neuronal activity in the central and peripheral nervous systems (Corder et al., 2018). An "affective-motivational" dimension of pain influences the aversive output in response to nociceptive input activation (Navratilova 2014). Our study sets out to examine the relationship in which endogenous opioids regulate this key pathway recognized in affective-motivational pain perception.

The medial thalamus plays a significant role in affective pain perception, in addition to the Anterior Cingulate Cortex (ACC) (Peyron et. al 2000). Chronic pain in rodents stems from hypersensitivity in the response of mediodorsal thalamus (MThal) to sensory stimuli. Neurons from the medial thalamus send glutamatergic afferents into various cortical areas, including but not limited to: the prefrontal cortex, ventral and dorsal anterior cingulate cortex, and the dorsomedial striatum (Hunnicutt et. al 2016). By projecting to the dorsomedial striatum, the ACC forms a circuit connecting the medial thalamus and the anterior cingulate cortex, which can both provide convergent properties via glutamatergic inputs to the dorsomedial striatum. Throughout the years, this thalamo-cortico-striatal circuit has been shown to involve key patterns in pain processing, particularly pain perception (Zhang et al 2015, Yokota et al 2016).

Mu-opioid receptors (MORs) and delta-opioid receptors (DORs) are predominantly expressed in the MThal and ACC (Mansour et. al 1994; Erbs et. al 2015). Injection of opioids into the MThal or ACC were shown to relieve pain in a previous animal model (Navritalova et. al 2015), suggesting a major role for opioid modification of thalamic and cortical circuitry in interpretive pain. The anterior cingulate cortex is augmented with MORs and DORs, as well as the endogenous opioid ligand enkephalin (Tanaka et al., 1994). Furthermore, the release of

opioid ligand enkephalin contributes greatly to the net effect of the thalamo-cortico-striatal circuit, contributing to modulation of rewarding behavior and pain based on response in the anterior cingulate cortex. (Birdsong et al., 2019).

Recent evidence shows the significance of opioids' modulatory properties in this circuit, yet evidence for endogenous opioids' role has remained unclear. Various research has been performed using these techniques, yet clear evidence based on these endogenous opioid's binding affinity and a resulting computational model has yet to be explored. Here, we present an additive understanding of the thalamo-striatal-cortical circuit's modulatory effects by exploring one of its key components.

Methods

We used ligand binding assays in the anterior cingulate cortex to measure affinity of endogenous opioid enkephalin and its displacement with non-selective agonist morphine in MORs, and cannabidiol in DORs, in order to examine the point at which activation occurs. Our expectation based on previous work shown in Kathman et. al is that enkephalin will show high binding affinity, and strong displacement at 10 nM concentrations.

We implemented whole cell recordings of pyramidal LV neurons in the anterior cingulate cortex, based on input from current injection in both MSN Str and MThal neurons to determine excitatory or inhibitory contribution based on endogenous ligand enkephalin concentration, 1nM and 10 nM. Our expectation is that there will be differing excitatory/inhibitory effects based on concentration.

We created a simple perceptron to simulate dual whole cell recordings and the thalamo-cortico-striatal circuit's learning. The perceptron was initialized such that MThal and MSN Str represented two input neurons and ACC neurons represented output. The neural network was trained using data from the previous experiment.

Results

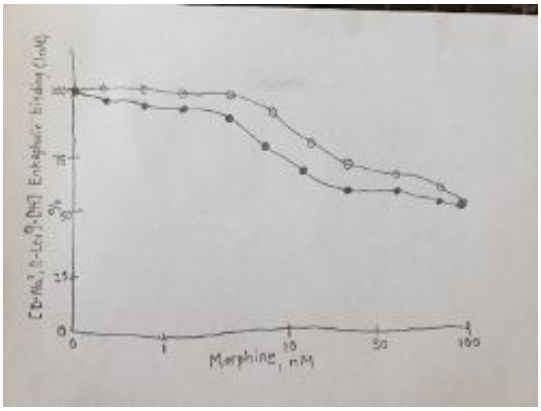

Figure 1A: Displacement curve of enkephalin by morphine phosphate. Rat brain slices were prepared and treated with no drug (black) or naloxone (white) and assayed with enkephalin (1nM) and increasing concentrations of morphine. Points represent each experiment.

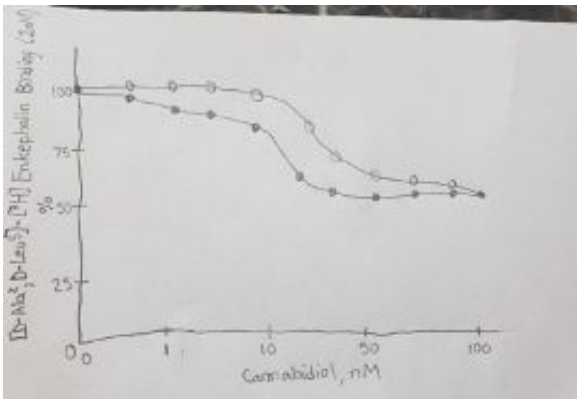

Figure 1B: Displacement curve of enkephalin by cannabidiol. Mouse brain slices were prepared and treated with no drug (black) or naloxone (white) and assayed with enkephalin (1nM) and increasing concentrations of cannabidiol. Points represent each experiment.

In order to find the specific concentration of endogenous opioid that will trigger activation in the thalamo-cortico-striatal circuit, we replicated previous experimentation based on the work of Kathman et. al at the microcosmic level. The experiment gives us an indicator of how strongly endogenous opioids bind to mu-opioid receptors in the brain, and thus provides insight for further experimentation at the level of the circuit. We began investigating binding affinity of endogenous enkephalin to MORs, by comparing morphine, a non-selective highly affinitive MOR agonist, with [D-Ala^2, DLeu^3]-[^{3}H]-enkephalin in the ACC mouse brain slice without drug and washed with naloxone, a highly selective opioid receptor antagonist. (Fig 1A). Initial displacements were seen at modest concentrations of morphine phosphate, 1 nM, followed by decreasing displacement as concentration of non-selective agonist morphine increased. The small amount of displacement at low concentration of endogenous ligand suggests high binding affinity of endogenous enkephalin on MORs.

As binding affinity of endogenous enkephalin to DORs may differ, we compared cannabidiol, a non-selective highly affinitive DOR agonist, with [D-Ala^2, DLeu^3]-[^{3}H]-enkephalin in the ACC mouse brain slice, again with no drug as well as washed with naloxone (Fig 1B). Similar features of displacement were seen compared to that of morphine in MORs as cannabidiol concentration increased, suggesting similar high binding affinity at lower agonist concentration. Crucially, the graph indicates that at high concentrations of agonist, displacement occurs in the receptors. Our next step was to increase the concentration of endogenous opioids and measure the effect at the level of the circuit.

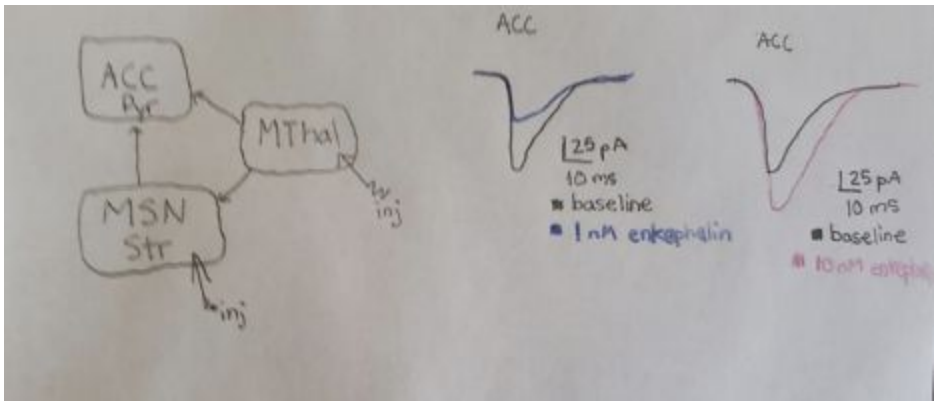

Figure 2: Whole-cell recordings from LV pyramidal neurons in the ACC in response to controlled stimulation of thalamus and striatal neurons, injected with 1 nM and 10 nM enkaphelin respectively.

Since previous results showed that enkephalin has high binding affinity in two major subtypes of opioid receptors, and high displacement was shown at the level of 10 nM concentration of agonist, we set out to determine from a systems perspective the net effect of endogenous release concentrations on the thalamo-striatal-cortical circuit. We sectioned coronal mouse brain slices and isolated striatal medium spiny neurons, layer V pyramidal ACC neurons,

and medial thalamus neurons (Figure 2). We injected both 1 nM and 10 nM enkephalin into this circuit and measured current response via LV pyramidal ACC neurons. We found that 1nM enkephalin binding causes an inhibitory net effect, while 10 nM enkephalin causes an excitatory net effect.

To better understand the thalamo-striatal-cortical circuit's modulatory effects in computation, a neural network was employed. A neural network is a representation of the human brain, with interconnected neurons forming an artificial network (Arnx, 2019). The functionality of a neural network is simple: it takes inputs as variables, does some calculation to them, and returns an output. In the context of this project, we wanted to quantify the thalamo-striatal-cortical circuit's modulatory effects in relation to enkephalin concentration in certain neurons. The neural network we created took in concentrations of enkephalin (in nM) in two input neurons (MThal and MSN Str) and the result was whether the circuit would produce an excitatory or inhibitory effect depending on these concentrations.

We programmed a single-layer perceptron with a learning rate of 1 based on the expected speed of the computation of neurons in this circuit, and a bias of 1 to shift the activation function by adding a constant to the input. We chose the Heaviside step function as our activation function as it most closely simulates a hypothetical action potential within neurons of this circuit, and set training iterations to 60.

Our code is viewable on Google Colab with a LionMail account.

MSN enkephalin injection conc., nM	MThal enkephalin injection conc., nM	ACC output (Excitatory or Inhibitory)
0.5	0.1	Inhibitory
0.1	1	Inhibitory
1	5	Inhibitory
5	1	Inhibitory
10	10	Excitatory
15	20	Excitatory
20	5	Excitatory
100	100	Excitatory
120	60	Excitatory

Figure 3: Shows neural network results when provided with certain concentrations of enkephalin in input neurons.

In Figure 3, we see evidence for this affective-motivational potential to learn the conditions in which to fire excitatory or inhibitory signals based on the input of endogenous opioid concentration. Based on our previous experiment, 1 nM enkephalin concentration caused an inhibitory effect and 10 nM enkephalin caused an excitatory effect. These values were then used to train the neural network. The value of 10 nM enkephalin concentration was used as the activation threshold so that the perceptron has to compute between 1 and 10 in order to determine whether to fire. Thus, for the edge cases of 1-10 nM enkephalin concentration in MThal and MSN Str input neurons, more nuanced exploration needs to be completed in order to present conclusive results, but these preliminary values were measured.

Discussion

Endogenous opioids in the anterior cingulate cortex showed high binding affinity in the two major subtypes of MORs and DORs. Homogenous binding affinity of opiate receptors in the anterior cingulate cortex shows that endogenous opioids likely affect ACC neurons similarly across subtypes, and showed possible inputs to explore via whole cell recordings.

Our hypothetical low to high concentrations of endogenous enkephalin have significantly different effects on the thalamo-striatal-cortical circuit, suggesting that endogenous opioid release has robust modulatory effects in reward learning and pain behavior. This data is very much dependent on implementing in-person procedures in the laboratory setting. In real world circumstances, we may find data that shows 1 nM enkephalin producing a smaller excitatory effect in comparison to 10 nM enkephalin, or both producing an inhibitory effect. Yet for the purposes of this course, the experiment represents a malleable proposition to serve as a stepping stone for the circuit's computation.

The perceptron we created in Figure 3 is the first step towards a computational understanding of the circuit's modulatory properties. We saw results consistent with our previous experiments that this simple circuit could learn based on the inputs given, but this is subject to modification based on the real world results needed from our second experiment.

Future experiments that include the measurement of different endogenous opioid subtypes would provide more robust evidence for the "affective-motivational" circuit as outlined. In addition, more nuanced stimulation of thalamal and striatal components will provide vital information for answering more questions for the entire circuit, but the results shown have major ramifications about the role of opioids in neurobiological functions. Finally, a neural network

with more complex functional components, including a backpropagation algorithm, can provide the scientific research community with a broader view of downstream effects in the thalamo-cortico-striatal circuit. Future experiments could add additional layers to the neural network to further elucidate the effects of endogenous opioids on the inhibitory/excitatory properties of the circuit. These results, though incomplete under the circumstances, represent an additive understanding of the role endogenous opioids could play in the “affective-motivational” circuit previously explored.

References

A. Mansour, C.A. Fox, S. Burke, F. Meng, R.C. Thompson, H. Akil, S.J. Watson (1994). Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. The Journal of Comparative Neurology 350:412–438.

E. Erbs, L. Faget, G. Scherrer, A. Matifas, D. Fillio, J.L. Vonesch, M. Koch, P. Kessler, D. Hentsch, M.C. Birling, M. Koutsourakis, L. Vasseur, P. Veinante, B.L. Kieffer, D. Massotte (2015). A mu-delta opioid receptor brain atlas reveals neuronal co-occurrence in subcortical networks. Brain Structure and Function 220:677–702.

E. Navratilova, J.Y. Xie, A. Okun, C. Qu, N. Eyde, S. Ci, M.H. Ossipov, T. King, H.L. Fields, F. Porreca (2012). Pain relief produces negative reinforcement through activation of mesolimbic reward-valuation circuitry. PNAS 109:20709–20713.

E. Tanka, A. North (1994). Opioid Actions on Rat Anterior Cingulate Cortex Neurons in vitro. The Journal of Neuroscience 14(3):1106-1113.

G. Corder, DC Castro, MR Bruchas, G Scherrer (2018). Endogenous and exogenous opioids in pain. Annual Review of Neuroscience 41:453–473.

Hunnicutt, Barbara J., et al. "A Comprehensive Excitatory Input Map of the Striatum Reveals Novel Functional Organization." *ELife*, edited by David C Van Essen, vol. 5, eLife Sciences Publications, Ltd, Nov. 2016, p. e19103. *eLife*, doi:10.7554/eLife.19103.

Navratilova, E., Porreca, F. Reward and motivation in pain and pain relief. Nat Neurosci 17, 1304–1312 (2014). https://doi.org/10.1038/nn.3811

R. Peyron, B. Laurent, L. García-Larrea, Functional imaging of brain responses to pain. A review and meta-analysis (2000), Neurophysiologie Clinique/Clinical Neurophysiology, Volume 30, Issue 5, 2000, Pages 263-288, ISSN 0987-7053, https://doi.org/10.1016/S0987-7053(00)00227-6.

W.T. Birdsong, B.C. Jongbloets, K.A. Engeln, D. Wang, G. Scherrer, T. Mao (2019). Synapse-specific opioid modulation of thalamo-cortico-striatal circuits. eLife 2019;8:e45146.

Yokota, Eiko, et al. "Opposite Effects of Mu and Delta Opioid Receptor Agonists on Excitatory Propagation Induced in Rat Somatosensory and Insular Cortices by Dental Pulp Stimulation." *Neuroscience Letters*, vol. 628, Aug. 2016, pp. 52–58. *ScienceDirect*, doi:10.1016/j.neulet.2016.05.065.

Zhang, Zizhen, et al. "Role of Prelimbic GABAergic Circuits in Sensory and Emotional Aspects of Neuropathic Pain." *Cell Reports*, vol. 12, no. 5, Elsevier, Aug. 2015, pp. 752–59. *www.cell.com*, doi:10.1016/j.celrep.2015.07.001.

Kathmann, Markus, et al. "Cannabidiol Is an Allosteric Modulator at Mu- and Delta-Opioid Receptors." Naunyn-Schmiedeberg's Archives of Pharmacology, vol. 372, no. 5, Feb. 2006, pp. 354–61. DOI.org (Crossref), doi:10.1007/s00210-006-0033-x.