

VIP Interneurons: A Key to Cortical Network Regulation in Avoidance Behaviour

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This review highlights and summarises the investigations of avoidance behaviour published in the journal *Neuron*. In the 6th issue of *Neuron*, Lee et al. (2019) makes an important link between the activity of prefrontal vasoactive intestinal polypeptide interneurons and their contribution to the initiation of avoidance behaviour in mice. Their role in hippocampal-prefrontal cortex communication networks allows the integration of specific-inputs to drive complex behaviours which are effectively lost following interneuron inhibition. This review of neurological experimentation aims to recognise the significant role of interneurons in cognitive thought and decision-making patterns like that seen in avoidance behaviour. The ability to avoid certain environments due to foreseeable pressures or potential harm is an evolutionary process that is not yet fully known. Therefore, understanding the cognitive process can help us learn how the brain formulates impressions and judgement in certain environments.

Abbreviations: mPFC – medial prefrontal cortex; VIP– Vasoactive intestinal polypeptide; vHPC – ventral hippocampus

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Introduction

Vasoactive intestinal polypeptide (VIP) interneurons have an inhibitory role in numerous cortical pathways. These nerve cells are involved in the excitation of pyramidal neurons via the inhibition of somatostatin neurons and enhance neural circuit performance for working memory in the medial prefrontal cortex (mPFC) (Ferguson & Gao, 2018). New studies support the role of VIP interneurons in the disinhibition of cortical excitatory neurons, allowing prefrontal responses to become influenced by hippocampal inputs. Therefore, these interneurons play a significant role in the initiation of functions such as avoidance behaviour (Lee et al., 2019). Monosynaptic projections originating from the ventral hippocampus (vHPC) are received by the mPFC to produce excitatory synaptic responses. This is supported by previous electrophysiological studies which indicate the

PFC and hippocampus have correlated activity (Sigurdsson & Duvarci, 2016). The hippocampus is responsible for memory formation, spatial navigation and roles in anxiety-related behaviours whereas the PFC is involved in executive functions like decision making (Huntley et al., 2020; Teffer & Semendeferi, 2012).

This review of interneuron function in cortical pathways aims to highlight their critical role in bodily functions, like avoidance behaviour, through the emergence of novel findings in the field of neuroscience. The work of Lee et al. (2019) seeks to investigate how behaviour is regulated via modulation of neural circuits using a multi-step experimental process. As this concept is not yet fully understood, their research is one of the pioneering investigations for this type of cognitive behaviour. Avoidance behaviour is the evolutionary action of an individual to avoid or escape an uncomfortable or endangering situation which may evoke pressure

or potential harm. Understanding the cognitive process can help us learn how the brain formulates decision making in certain environments.

Recent articles have described the role of certain neuronal regions in the development of avoidance behaviour. For example, these distinct behaviours are shown to positively correlate with the synchronisation of neural oscillations. Specifically, an increase in theta-frequency oscillatory synchrony between the vHPC and mPFC is produced during avoidance activity (Padilla-Coreano et al., 2019; Harris & Gordon, 2015). This suggests a direct relationship between hippocampal-prefrontal circuits and the establishment of certain behaviours as well as evidence for the role of synchronised oscillations in neural transmission. In addition to avoidance behaviour, other functions identified that are regulated by this interaction include spatial working memory and associative learning (Sin et al., 2019). While the physiological processes behind these behaviours are well established, the mechanisms by which interneurons work are not yet fully understood (Cardin, 2018).

Experimental Methodology

In this issue of *Neuron*, Lee et al. (2019) investigated how prefrontal VIP interneuron activity initiates avoidance behaviour within the hippocampal-prefrontal cortical circuit. This was achieved by examining whether mice would choose to either avoid or explore the open arms of an elevated plus maze (EPM). The EPM consists of two sheltered 'closed arms' and two brightly lit 'open arms.' Open arms create a more threatening environment to encourage avoidance behaviour (Lee et al., 2019). Fibre photometry with GCaMP6 fluorescence was used to measure VIP interneuron activity in the mPFC. Higher VIP-GCaMP signals were observed in mice when placed in the open arms compared to the closed arms, suggesting more VIP interneuron activity. The authors drew two potential theories from these observations. Either VIP interneurons drive

open arm exploration or trigger anxiety-related signals to encourage avoidance behaviour (Lee et al., 2019). To investigate whether high VIP interneuron activity encourages avoidance or explorative behaviour, the actions of mice to explore the open or closed arms of the EPM were observed.

The authors tested the extent of this relationship directly using optogenetics. By implanting bilateral fibre optics into the mPFC of mice injected with Cre-dependent archaerhodopsin (Arch), VIP interneurons were successfully inhibited *in vitro* (VIP-Arch). Mice injected with an enhanced yellow fluorescent protein (eYFP) retained interneuron function. Further trials were conducted to quantify light-induced changes of behaviour in light ON and OFF epochs. A stimulatory light was delivered to mice in the open arms of the EPM. The first trial was conducted with a light ON epoch (Lee et al., 2019).

Only male mice older than four weeks were used for experimentation which involved optogenetic inhibition or fibre photometry by Lee et al. (2019). Both female and male mice were used for experiments that combined optogenetic inhibition and microendoscopic GCaMP imaging, however, the effects of gender were not analysed due to a limited group size.

Results and Discussion

Lee and colleagues (2019) hypothesised vHPC input is recruited by VIP interneurons to control prefrontal responses. This was tested by stimulating channelrhodopsin-2 (ChR2) in vHPC terminals and recording pyramidal neuron activity from acute mPFC slices. Subsequent excitatory postsynaptic currents in VIP interneurons were produced following optogenetic-stimulation of vHPC terminals. This showed a causal relationship between hippocampal input and VIP interneuron stimulation. Furthermore, mPFC excitation from vHPC input was reduced following VIP interneuron inhibition (Lee et al., 2019). Trial

repeats showed VIP interneuron inhibition increased inhibitory synaptic currents in the mPFC but produced no effect on excitatory currents.

Results and observations from experimentation show that a lower VIP-GCaMP signal predicted a higher incidence of open arm exploration whereas a higher VIP-GCaMP signal predicted a lower incidence of open arm exploration (Lee et al., 2019). These results suggest VIP interneuron activity is involved in decisions regarding initiation of avoidance behaviour. Results show VIP-Arch mice demonstrated a substantial increased time in the open arm of the EPM compared to VIP-eYFP mice. Secondly, VIP-Arch mice spent significantly more time in the open arms during the ON epoch compared to the OFF epoch. Thirdly, time spent in the light ON epoch was significantly less than the light OFF epoch for VIP-eYFP controls (Lee et al., 2019).

As VIP interneurons disinhibit hippocampal-prefrontal inputs, which process information of the mouse's environment, the authors examined whether VIP interneurons are required for prefrontal representations of open vs closed arms. Prefrontal network activity encoding open vs closed arm was identified using a dual colour micro-endoscope with GCaMP imaging of mPFC activity and eNpHR activation in VIP interneurons (Lee et al., 2019). GCaMP signal correlation matrixes were computed to characterise network-level activity patterns, and data were classified depending on whether the mice were in the open or closed arms. Patterns of prefrontal network activity was then determined. Lee and colleagues found that in mice with inhibited VIP interneurons, there were minimal differences in prefrontal activity between open and closed arms. This correlation was also seen in control groups, which possessed a higher magnitude of mPFC activity as seen with higher

GCaMP signals (Lee et al., 2019). Therefore, it is clear that VIP interneuron inhibition disrupts network level representations of positioning in the open or closed arms as indicated by the suppression of GCaMP signals in VIP-Arch mice.

By showing that inhibition of prefrontal VIP interneurons attenuates prefrontal responses to hippocampal input, Lee et al. (2019) investigated if VIP interneuron inhibition contributing to open arm exploration is influenced by the strength of mPFC communication. The level of communication correlates with emitted theta frequency. In the study, VIP interneuron inhibited mice with high theta synchrony produced a marked increase in open arm exploration. However, when theta synchrony was low, inhibition did not increase open arm exploration. As theta synchrony can be used as a biomarker for anxiety-related signalling in the brain, interneurons enhance transmission of this input to promote avoidance behaviour (Jacinto et al., 2016). Low theta synchrony means weak vHPC input to mPFC. Therefore, interneuron inhibition cannot alter avoidance behaviour with behavioural effects being determined by the state of hippocampal-prefrontal signalling. The initiation of avoidance behaviour in mice is illustrated in Figure 1.

Despite measures employed to maintain result accuracy, some limitations still exist. While we share similar chemistry with mice, applications of these findings (i.e., Lee et al., 2019) may not be transferable to humans. Therefore, similar experiments should be performed on humans to ensure equivalency of results. Additionally, trial repeats were not performed for most experiments which decreases the overall reliability of trends produced. Multiple trials of each experiment must be performed to produce more accurate data and more reliable conclusions.

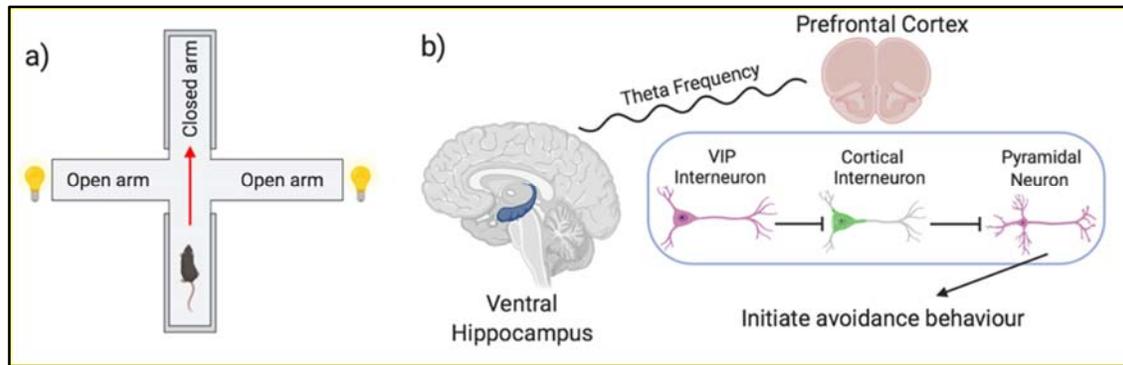


Figure 1: Cortical initiation of avoidance behaviour. A mouse avoids the exposed open arms of the EPM and progresses to the safer closed arm (A). Input from the ventral hippocampus of high theta frequency is directly received by VIP interneurons in the prefrontal cortex. This inhibits other inhibitory interneurons in the cortex to initiate pyramidal neuron excitation (B). Prefrontal responses of avoidance behaviour are therefore stimulated to prevent exploration of the open arms.

To summarise, Lee et al. (2019) uncovers the mechanisms by which VIP interneuron disinhibition of cortical activity contributes to avoidance behaviour. Where VIP interneuron activity increased in mice in the open arms, levels help predict future events of exploration or avoidance behaviour. If VIP interneuron activity was high, mice were more likely to avoid the open arms compared low activity. Artificial inhibition of VIP interneurons resulted in a significant decrease in open arm avoidance. Additionally, with high hippocampal-prefrontal theta synchrony, VIP interneurons disinhibit prefrontal responses to hippocampal input (Lee et al., 2019). These results demonstrate cortical circuits driving avoidance behaviour are enabled by VIP interneurons. The ability to gate hippocampal inputs that are transferred as prefrontal responses means these interneurons are essential in the decision-making process. This research holds particular significance due to the new understanding it brings in pathophysiological mechanisms. Where previous studies investigated VIP interneuron disinhibition at a single-neuron level, Lee et al. (2019) reveals insights into the function of interneurons in the entire cortical network (Ayzenshtat et al., 2016; Karnani et al., 2016). For example, the level of VIP interneuron involvement in cortical circuits can be used as a biomarker for subsequent behavioural changes. Furthermore, the authors identified that

behavioural effects depend on the state of the hippocampal-prefrontal network. This indicates that interneurons cannot directly manipulate cells to cause avoidance behaviour. However, it is clear cortical circuits are enabled by interneuron activity as hippocampal inputs become integrated into specific network-level representations.

Future Directions and Conclusion

In reviewing the experimental investigation of Lee et al. (2019) published in the journal *Neuron*, consequences from the disinhibition and inhibition of VIP interneurons have highlighted their key role in avoidance behaviour by observing the actions of mice. This helps to uncover the intricate network of neurons which are involved in cognitive behaviour and decision making in pressured environments.

Despite the significance of the findings from this article, the study has limitations. Results represent cortical functions in mice and cannot represent the exact function of human brains. Further research is required to ensure neural circuits associated with avoidance behaviour can be replicated in human trials. This can be done by simulating similar neuronal tracing methods and producing an environment that replicates an open arm of the EPM. Functional magnetic resonance imaging could be used to capture specific regions of stimulation in the brain (Lynn et al., 2016). It

could identify other brain regions that may be involved in the initiation of avoidance behaviour. Surgical inhibition of the VIP interneuron in humans would remain unethical so this approach could provide a useful alternative. Additionally, stimuli with varying threat levels would be required in the EPM to compare the magnitude of avoidance behaviour initiated. Reliability of results would also increase after multiple trial runs. With a larger subject group size for trialling, a comparison of experimental effects on males and females could be reached. This comparison could be used to draw potential conclusions regarding structural differences between genders which may ultimately lead to altering behaviours. Other variables may be compared in trial groups to investigate similar effects.

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