a climbing fiber projection to the same part of its hemispheres and/or vermis (Figure 1), it is, in a way, surprising that it took the field so long to address these questions. The reason why we had to wait for the heroic experiments by Garcia-Garcia and colleagues[1] is probably because these experiments form technically a tour de force in that calcium transients of different cell types distributed across different cell layers of the same area needed to be measured at the same time in awake, behaving animals that were subjected to a long-term training paradigm involving multiple time intervals. Their unique datasets indicate that the mossy fiber-granule cell system and climbing fiber system together determine the onset, duration, and offset of the different stages of the behavior involved and that together they can flexibly adapt to the environmental demands. By showing this for reward learning, the authors encourage neuroscientists to explore the same principles for other behaviors (Figure 1). Additionally, they pave the way for the next avenue of research, which is to study activity of the cerebellar cortex together with that of its upstream and downstream areas, so as to elucidate how the common sources of the mossy fiber and climbing fiber systems may drive them to optimize interval timing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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The lateral septum returns to the center stage of brain reward

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https://doi.org/10.1016/j.neuron.2024.07.012

In this issue of *Neuron*, Chen et al.[1] found that lateral septum Esr1-expressing cells respond to both non-drug and drug rewards. Mice will lever press for optogenetic stimulation of these neurons, which are also critical to methamphetamine locomotor sensitization, conditioned place preference, self-administration, and reinstatement.

In 1954, Olds and Milner[2] implanted 15 male rats with electrodes in their brains and placed them in Skinner boxes to determine if lever pressing for electrical stimulation would serve as an operant reinforcer (increasing the probability of a response compared with no stimulation), operant punisher (decreasing the probability of a response), or neutral (neither increasing nor decreasing). Of the 15 rats, 4 with electrodes located in the lateral septum (Figure 1) and 1 with electrodes located in the mammillothalamic tract significantly increased lever pressing when the stimulation was ON and decreased lever pressing when the stimulation was OFF (the "best" septal rat lever-pressed on average 742 times per hour).

This seminal study, widely regarded as the inspiration for a new era of exploring brain mechanisms of reward, has led to

decades of follow-up studies on the so-called "brain reward system."[3] This research has identified many brain areas supporting intracranial electrical self-stimulation and, following the introduction of optogenetic and chemogenetic methods, identified many cell types and neuronal projections that support reinforcing brain stimulation.[4–6] In parallel, early studies in the 1970s–1980s using classical pharmacology and lesion methods identified a critical role of the mesocorticolimbic dopamine system in the reinforcing effects of drug and non-drug rewards.[3] Based on this knowledge, studies using traditional electrical stimulation methods and newer optogenetic and chemogenetic methods have primarily focused on this system and its efferent and afferent projections.[4,5] Consequently, while the lateral septum was the first structure reported to support brain stimulation reward, its role in the reinforcing effects of drug and non-drug rewards remains largely unknown, with a few sporadic exceptions over the years (e.g., Prado-Alcala et al., Luo et al., and Pantazis et al.[7–9]).

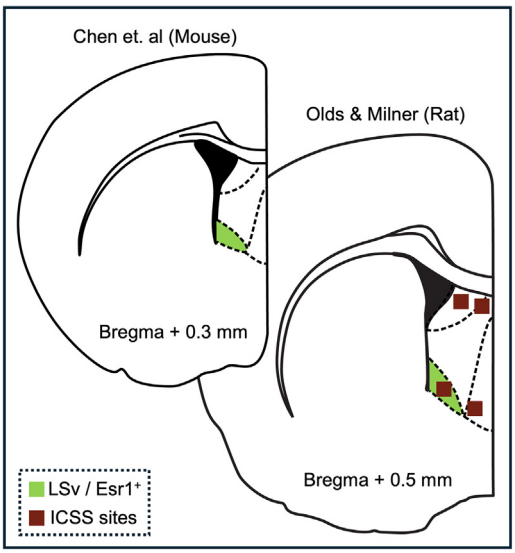

Figure 1. Graphical presentation of the approximate location of lateral septum ESR1-expressing cells in mice in the study of Chen et al. and the approximate location of lateral septum electrodes in rats in the study of Olds and Milner

LSv, ventral part of the lateral septum; ICSS, intracranial self-stimulation.

Against this background, Chen et al.[1] used cutting-edge neuroscience methods in transgenic mice to investigate the spatial arrangement, connectivity, and function of lateral septum cell types and projections in reward-related processes, including the rewarding effects of the addictive drug methamphetamine. The authors used single-nucleus RNA sequencing to map the genetic diversity of cells within the lateral septum and spatial transcriptomic approaches to visualize the spatial distribution of the identified cell types. Based on these analyses, they chose three GABAergic clusters for measurement of neuronal activity and functional manipulations: somatostatin-, neurotensin-, and estrogen receptor 1 (Esr1)-expressing cells (Figure 1). These cell types represent largely non-overlapping cells and exhibit a gradient distribution along the lateral septum dorsal-to-ventral axis.

The authors reported that Esr1-expressing cells, but not somatostatin- or neurotensin-expressing cells, were activated during exposure to chocolate and social interaction and after acute methamphetamine injections. Additionally, mice nose-poked for optogenetic stimulation of Esr1-expressing cells and showed a preference for a context paired with this stimulation. These effects were not observed after stimulation of somatostatin- or neurotensin-expressing cells.

Next, the authors used circuit mapping methods and showed that lateral septum Esr1-expressing cells send dense projections to the ventral tegmental area (VTA), the cell body region of the mesocorticolimbic system. Additionally, as with direct stimulation of lateral septum Esr1-expressing cells, optogenetic stimulation of lateral septum Esr1-to-VTA projection supported nose-poke response and induced place preference. The authors then showed that the rewarding effects of stimulation of the lateral septum Esr1-to-VTA projection are due to direct inhibition of VTA GABAergic neurons, leading to a disinhibition of VTA dopamine neurons and increased dopamine release in the nucleus accumbens.

Overall, these data indicate that artificial stimulation of lateral septum Esr1-expressing cells is rewarding, and that the likely mechanism is disinhibition of the VTA dopamine projection to the nucleus accumbens.

Next, the authors determined the role of lateral septum Esr1-expressing cells in the rewarding effects of chocolate, social interaction, and methamphetamine-induced locomotor sensitization, methamphetamine self-administration, and cue-induced reinstatement of methamphetamine seeking after extinction of the drug-reinforced responding. They showed that selective blockade of synaptic transmission of Esr1-expressing cells (using tetanus toxin light chain) decreased methamphetamine self-administration, conditioned place preference (CPP), and methamphetamine-induced locomotor sensitization. In contrast, the same lesion manipulation had no effect on palatable food (liquid Ensure) self-administration or social preference.

In other experiments, they showed that chemogenetic inhibition of lateral septum Esr1-expressing cells decreased cue-induced reinstatement of methamphetamine seeking. The authors also found that non-contingent methamphetamine exposure increased the expression of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in lateral septum Esr1-expressing neurons, leading to increased excitability. Additionally, Esr1-specific Hcn1-short hairpin RNA knockdown decreased methamphetamine-induced locomotor sensitization. However, these data may not be relevant to methamphetamine reward, because the same manipulation had no effect on methamphetamine CPP.

From an addiction perspective, a limitation of the study is the use of behavioral procedures (limited access drug self-administration and cue-induced reinstatement) whose prospective predictive validity in identifying medications for drug addiction has not been established.[10] Thus, a question for future research is whether lateral septum Esr1-expressing cells also contribute to drug taking and drug seeking in animal models that more closely mimic the human condition, like drug self-administration despite adverse consequences and choice between drugs and non-drug rewards.

In conclusion, Chen et al. demonstrated that lateral septum Esr1-expressing cells are activated by both non-drug (palatable food and social interaction) and drug (methamphetamine) rewards. Optogenetic stimulation of these neurons is highly rewarding, likely through activation of the VTA-to-nucleus accumbens dopamine projection. They also established a causal role for these cells in methamphetamine locomotor sensitization, CPP, self-administration, and cue-induced reinstatement of drug seeking but surprisingly not in palatable food (Ensure) self-administration or social preference. These novel findings are poised to reposition the lateral septum at the forefront of research on brain reward mechanisms, 70 years after Olds and Milner's pioneering discovery that rats will lever press for electrical stimulation in this region. While James Olds (May 30, 1922–August 21, 1976) and Peter Milner (June 13, 1919–June 2, 2018) are no longer with us, their groundbreaking work continues to inspire, and they would likely be very impressed by Chen et al.'s exceptional study.

ACKNOWLEDGMENTS

The commentary was supported by funds to the Intramural Research Program of NIDA (ZIA-DA000434, Y.S.). The authors used ChatGPT on the original draft of the text to identify grammar errors and improve the clarity of the text. The content of the text remained intact.

AUTHOR CONTRIBUTIONS

The authors contributed equally to the write up of the preview.

DECLARATION OF INTERESTS

The authors declare no competing financial interests.

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