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Priority Communication

Glutamatergic Ventral Pallidal Neurons Modulate Activity of the Habenula–Tegmental Circuitry and Constrain Reward Seeking

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ABSTRACT

BACKGROUND: The ability to appropriately integrate and respond to rewarding and aversive stimuli is essential for survival. The ventral pallidum (VP) plays a critical role in processing both rewarding and aversive stimuli. However, the VP is a heterogeneous structure, and how VP subpopulations integrate into larger reward networks to ultimately modulate these behaviors is not known. We identify a noncanonical population of glutamatergic VP neurons that play a unique role in responding to aversive stimuli and constraining inappropriate reward seeking.

METHODS: Using neurochemical, genetic, and electrophysiological approaches, we characterized glutamatergic VP neurons (n = 4–8 mice/group). We performed patch clamp and in vivo electrophysiology recordings in the lateral habenula, rostromedial tegmental nucleus, and ventral tegmental area to determine the effect of glutamatergic VP neuron activation in these target regions (n = 6–10 mice/group). Finally, we selectively optogenetically stimulated glutamatergic VP neurons in a real-time place preference task and ablated these neurons using a virally expressed caspase to determine their necessity for reward seeking.

RESULTS: Glutamatergic VP neurons exhibit little overlap with cholinergic or gamma-aminobutyric acidergic markers, the canonical VP subtypes, and exhibit distinct membrane properties. Glutamatergic VP neurons innervate and increase firing activity of the lateral habenula, rostromedial tegmental nucleus, and gamma-aminobutyric acidergic ventral tegmental area neurons. While nonselective optogenetic stimulation of the VP induced a robust place preference, selective activation of glutamatergic VP neurons induced a place avoidance. Viral ablation of glutamatergic VP neurons increased reward responding and abolished taste aversion to sucrose.

CONCLUSIONS: Glutamatergic VP neurons constitute a noncanonical subpopulation of VP neurons. These glutamatergic VP neurons increase activity of the lateral habenula, rostromedial tegmental nucleus, and gamma-aminobutyric acidergic ventral tegmental area neurons and adaptively constrain reward seeking.

Keywords: Aversion, Dopamine (DA), Electrophysiology, Gamma-aminobutyric acid (GABA), Rostromedial tegmental nucleus (RMTg), Ventral tegmental area (VTA)

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Adaptively modulating reward seeking and responding to aversive stimuli are essential to survival. Reward seeking despite negative consequences is a hallmark feature of mood and substance use disorders (1–4). These disorders have been associated with altered activity and function within the reward system, composed of dopamine (DA) neurons in the ventral tegmental area (VTA) and its projections such as the nucleus accumbens (NAc). The ventral pallidum (VP) is a critical node in this network, being a primary output of the NAc (5) and projecting to the VTA, lateral habenula (LHb), and thalamic nuclei (1,6,7). The VP was first identified as a limbic motor interface (8,9) necessary for the expression of motivated behavior and for hedonic processing of reward (6,10). In humans and rodents, the VP is activated in response to cues predicting reward (11–13), while VP lesions decrease reward motivation and hedonic value (14–17). The VP also processes aversive stimuli and has been proposed to integrate positive and negative affective information to guide behavior (18–20). With its central role in the reward network, modulation of the VP has been explored as a therapeutic strategy in psychiatric disease (21). However, to rationally manipulate VP function, comprehensive understanding of its heterogeneity, subcircuitry, and function in reward-related behavior is needed.

The VP is a predominantly gamma-aminobutyric acidergic (GABAergic) nucleus (22) with cholinergic neurons. Within these neurochemical identities, VP neurons exhibit diverse morphological and membrane properties (23,24) as well as mixed electrophysiological responses to rewarding and aversive stimuli, suggesting functional heterogeneity (6). Vesicular glutamate transporter (VGluT2) messenger RNA (mRNA) is expressed in the VP (25,26), although it is unclear whether these VGluT2-positive neurons represent a distinct subclass or

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whether they overlap with canonical GABAergic or cholinergic neurons. Supporting the idea that subtypes of VP neurons play unique roles in reward-related behavior, it was recently reported that a subset of parvalbumin (PV)-positive VP projection neurons are capable of glutamate co-release and mediate behavioral changes following defeat stress (27). To date, it is not known how the described functional heterogeneity maps onto neurochemically defined populations of VP cells or how populations of glutamatergic VP neurons contribute to the expression of reward-related behavior.

Here, we characterized a glutamatergic subpopulation of VP neurons and investigated how these neurons integrate into the larger reward circuitry. Using patch clamp and in vivo electrophysiology, we showed that activation of glutamatergic VP neurons modulates firing rates of LHb and rostromedial tegmental nucleus (RMTg) neurons as well as GABA and DA neurons in the VTA. Strikingly, while nonspecific optogenetic stimulation of the VP induces a robust place preference, selective stimulation of glutamatergic VP neurons produces behavioral avoidance. Ablation of these neurons revealed their critical role in adaptively constraining reward seeking. Our results point to the possibility of modulating this population as a therapeutic strategy for disorders of maladaptive reward seeking.

METHODS AND MATERIALS

Animals

Adult male and female VGlut2-IRES-Cre, VGAT-IRES-Cre, ChAT-Cre, PV-Cre, and wild-type littermates on a C57BL/6J background were used (28–30). All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health, and with approval of the Institutional Animal Care and Use Committee at the University of Maryland. (See Supplement for experimental details.)

Stereotaxic Surgery

Adeno-assocated virus–expressing Cre-inducible channelrhodopsin-2 (ChR2)-enhanced yellow fluorescent protein (eYFP), mCherry, rabies virus, or taCasp was bilaterally injected into the VP and expressed for 28 days. Optic fibers, constructed in-house as described previously (31), were implanted bilaterally over the VP and secured with three skull screws and dental cement. Mice with verified viral infection sites and fiber placements in the VP were included in the analyses.

Patch Clamp Electrophysiology

Whole-cell patch clamp recordings were made of the VP, LHb, VTA, and RMTg. Neurons were recorded with cesium-based (voltage clamp) or cesium-free (current clamp) physiological internal solution. Currents were amplified, digitized, and analyzed using a MultiClamp 700B amplifier and Digidata 1440A and Clampex 10.7.0.3 software (Molecular Devices, Sunnyvale, CA).

In Vivo Electrophysiology

Mice were anesthetized, and craniotomies were made over the LHb, VTA, and RMTg. Silicone electrodes (NeuroNexus Technologies, Ann Arbor, MI) were lowered to the recording site. Blue light was delivered to the VP (1-second pulses, 10-second interstimulus interval). Voltage was recorded and analyzed using PlexControl, Offline Sorter, and NeuroExplorer (Plexon, Dallas, TX).

Behavioral Studies

Real-Time Place Preference. Mice first had ChR2 injected bilaterally in the VP, and optic fibers were implanted over the same site. Mice were then individually run in a behavioral arena with two experimental zones distinguished by floor texture and wall patterns. During the test, 4-ms light pulses were delivered at 1, 10, or 20 Hz when the mice entered the "active" zone; time in zones was analyzed with EthoVision software (Noldus Information Technologies, Leesburg, VA).

Operant Task. Mice were lightly food restricted before training on a fixed ratio (FR) (FR 1, 2, 3) task for sucrose pellets. Following acquisition, mice were tested for 6 days on an FR 5 and three progressive ratio (PR) sessions. Mice were then injected with AAV5-flex-taCasp-TEV to the VP and allowed 3 days of recovery following surgery. Testing identical to baseline sessions continued for a total of 10 days.

Taste Aversion. During habituation (3 days), mice were water deprived and given 30 minutes to consume tap water in the experimental arena. Water was then replaced with 5% sucrose solution; after 4 days baseline, mice were injected with lithium chloride (LiCl) (0.15 M, given intraperitoneally at 2% body weight) and were tested the following day. Drinking was recorded using infrared sensors (Arduino, Turin, Italy).

RESULTS

Glutamatergic VP Neurons Exhibit Distinct Neurochemical and Membrane Properties

We first determined whether glutamatergic VP neurons overlapped with canonical VP subtypes. We injected DIO-mCherry into the VP of *VGluT2-IRES-Cre* mice to visualize glutamatergic neurons (Figure 1A). The VP is predominantly GABAergic, with cholinergic and PV-positive neurons (22,32,33). We immunostained against PV or choline acetyltransferase (ChAT) and quantified overlap with mCherry-expressing neurons. PV was coexpressed in 17.5 ± 2.06% of VGluT2-positive neurons, and the extent of overlap varied as a function of anterior to posterior gradient, with highest colocalization occurring in the anterior VP (anterior 22.6 ± 4.6%, medial 19.7 ± 2.5%, posterior 9.2 ± 2.4%) (Figure 1B). ChAT colabeled with 4.3 ± 1.8% of VGluT2-positive neurons (Figure 1C).

Most VP neurons are GABAergic (12,22). To determine the extent of overlap between VGluT2-positive and GABAergic neurons, we performed fluorescent in situ hybridization to detect Slc17A6 (VGluT2) and Slc31A1 (vesicular GABA transporter [VGAT]) mRNA and quantified overlap of fluorescent signals. Slc31A1 mRNA was observed in very few (1.98 ± 0.66%) Slc17A6-expressing neurons (Figure 1D), suggesting sparse neurons capable of both glutamate and GABA release (Figure 1D).