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Research report

The role of neuropeptide CART in the lateral hypothalamic-ventral tegmental area (LH-VTA) circuit in motivation

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HIGHLIGHTS

- Intracranial self-stimulation (ICSS) was facilitated by CART.
- ICSS resulted in robust activation of CART cells in the LH.
- ICSS induced synaptic plasticity in CART system in reward circuit.
- Rats can be conditioned to self-administer CART in pVTA.
- CART neurons of LH-MFB may process motivation *via* the mesolimbic dopamine pathway.

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ABSTRACT

Rats with electrode implanted in the lateral hypothalamus (LH)-medial forebrain bundle (MFB) area actively engage in intracranial self-stimulation (ICSS). However, the neuronal substrate that translates the electrical pulses into the neural signals, and integrates the information with mesolimbic reward system, has remained elusive. We test the hypothesis that the cocaine- and amphetamine-regulated transcript (CART) neurons in the LH-MFB area may support this function. The ICSS activity *via* an electrode in LH-MFB area was facilitated by CART (55–102) peptide stereotaxically injected in the lateral ventricle or posterior ventral tegmental area (pVTA), but attenuated by CART antibody. While the ICSS experience seems to activate CART cells in the LH, the pVTA showed significant increment in the CART fiber terminals on the dopamine cells, increase in tyrosine hydroxylase (TH)-immunoreactivity, and CART and synaptophysin colabeled elements. Neuronal tracing experiments revealed that CART cells of the LH-MFB region project to the pVTA. The rats with stereotaxically implanted cannulae in pVTA avidly self-infused CART (55–102) suggesting a role for the peptide in motivation, however, CART (1–39) was ineffective. CART self-infusing activity was inhibited by dopamine D1 receptors antagonist, given directly in the nucleus accumbens shell (AcbSh). The rats trained to self-administer CART (55–102) showed enhanced TH immunoreactivity in the cells of pVTA and fibers in AcbSh. We suggest that CART neurons of the LH-MFB area may play a role in conveying reward information to the mesolimbic dopamine neurons, which in turn may arouse the goal directed behavior.

1. Introduction

The brain reward system and its capability to shape motivational behaviors are of prime importance to the survival of a species. Olds and Olds [1] demonstrated that medial forebrain bundle (MFB) is a neural site that supports high frequency intracranial self-stimulation (ICSS). The rats with an electrode in the lateral hypothalamic (LH)-MFB area incessantly engage in ICSS and the response has been attributed to the activation of myelinated descending fibers [2] that feed into the ventral tegmental area (VTA). From here emerges the dopaminergic mesolimbic pathway that innervates the nucleus accumbens (Acb) and prefrontal cortex, and plays a major role in shaping the reward and motivational behaviors [3,4]. The mesolimbic pathway can be stimulated

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by a number of pleasure giving agents like opioids, ethanol, nicotine which act at the level of posterior VTA (pVTA) [5,6], or by amphetamine or cocaine given directly in the nucleus accumbens shell (AcbSh) [7,8]. Studies aimed at understanding the interaction between these agents and the neuroanatomical substrates are important, since they provide insights into addiction, one of major afflictions of our society.

However, one major question remains. What is the precise neuronal substrate in the LH-MFB region that translates the incident electrical pulses into the neural signals and integrates with the dopaminergic mesolimbic system to produce reward? Available evidences implicate acetylcholine [9], glutamate [10], orexin [11,12] or melanocyte concentrating hormone (MCH) [13] containing neurons, but the data are inconclusive. Interestingly, LH-MFB region in the brain of rat shows a strong population of CART neurons. Additionally, CART, or its mRNA, was detected in the pVTA and AcbSh, the major reward supporting nodes of the brain [14,15]. The CART neurons in perifornical area of LH, project to AcbSh and VTA [16]. In terms of function, the peptide promotes conditioned place preference [17], interacts with psychostimulants [18], and its role in reward has been suggested [19].

In the present study, rats were implanted with an electrode in the LH-MFB area and trained to self-stimulate in an operant chamber. We tested if the CART peptide can modulate the threshold for lever press activity. The reliability and reproducibility of this technique in assaying reward is fully validated [20,21]. The ability to evoke self-infusion is a hallmark of a rewarding molecule. Using this operant paradigm, we show that the rats can be conditioned to self-infuse CART directly into the pVTA. We also find that the conditioning experience activated the CART neurons and up regulated the expression of CART and dopamine in the LH-pVTA-AcbSh circuit. Application of synaptophysin antibody, as a marker for synaptic protein, revealed that electrical self-stimulation experience may promote synaptic plasticity in CART neurons.

2. Materials and methods

2.1. Animals and surgery

Adult male Wistar rats (250–300 g) were anesthetized with a mixture of ketamine (90 mg/kg; Ketmin® 50, Themis Medicare Ltd., India) and xylazine (10 mg/kg; Xylaxin®, Indian Immunologicals Ltd., India) given *via* intraperitoneal (ip) route and placed in stereotaxic instrument (David Kopf Instruments, USA). For ICSS protocol, a set of rats was implanted with bipolar electrode, prepared in-house [22], into the right MFB (AP: −2.8 mm, ML: +1.7 mm, DV: −8.5 mm), and with a 24 gauge stainless steel guide cannula [23] in the lateral ventricle (AP: −0.8 mm, ML: −1.3 mm, DV: −3.5 mm) or pVTA (AP: −5.6 mm, ML: +2.1 mm, DV: −8.5 mm ventral from the surface of the skull at 10° angle to the vertical) [24] for delivery of the agents.

For CART self-administration experiments, rats were implanted with guide cannulae into the right pVTA (coordinates given above) or anterior VTA (aVTA; AP: −4.8 mm, ML: +2.1 mm, DV: −8.5 mm ventral from the surface of the skull at 10° angle to the vertical) in the first set of animals. Additional groups of animals were implanted with cannulae in pVTA and AcbSh (AP: +0.7 mm, ML: +1.0 mm, DV: −6.5 mm) [24] and used for pharmacological interventions. Following surgery, the rats were allowed 7 days to recover and then subjected to training in the operant chamber followed by behavioural assays as described below. All protocols were conducted during 09:00–13:00 h. The protocols were carried out under strict compliance of the Institutional Animal Ethics Committee.

2.2. Conditioning of the rats for ICSS

ICSS paradigm is a reliable tool to evaluate motivational aspects of the brain reward system [20,25–28] and we followed the procedure with slight modifications [29]. The rats implanted with stimulating electrode targeted at the LH-MFB area were individually placed in an operant chamber (Coulbourn Instruments, USA; 30.48 × 25.4 × 30.48 cm) having two retractable levers [25,26]. The rats were kept on a continuous reinforcement schedule fixed ratio 1 (FR1) to press the lever for electrical self-stimulation. The stimulator (Coulbourn) was connected to electrode *via* stimulating cable [22] and was controlled by software (Graphic State Notation-3.03). The rat was conditioned to press the lever in an operant chamber to self-stimulate LH-MFB area. Reward and reinforcement was evaluated in terms of the lever press activity, and represented as rate-frequency curves and ICSS threshold (M_{50} and T_0) [21,30]. The frequency that maintains 50% of maximal responding, is often called as 'half maximum' or M_{50} value. The frequency at which the stimulation becomes rewarding and where the line intersects the x-axis was considered as 'Theta-0′ (T_0) (rate of responding>0) [21,28,30,31]. The ICSS threshold (M_{50} and T_0) was calculated using nonlinear regression curve fit method using Graphpad Prism 5 software [30–33]. During ICSS conditioning, the cathodal current was used. The use of the cathodal current is appropriate not only because it allows conditioning at lower stimulation parameters but because prolonged application of anodal current may lesion the tissue. Furthermore, the intensity of stimulation current (∼100–300 μA) and the descending frequency range (∼186–33 Hz) used herein were similar to those employed in earlier studies [34]. In the ICSS rate-frequency conditioning, we got optimum lever press activity between 35 and 40 lever presses per 50-s which was similar to that reported in the previous studies [29,34]. Each ICSS conditioned rat was subjected to fifteen trials of descending series of frequencies of one min each. The frequency was varied instead of intensity of stimulation since such manipulations activated the constant population of neurons. The data were then converted into% ICSS threshold (M_{50} and T_0). The leftward shift in the rate-frequency curve function indicated reward facilitation, while the rightward shift suggested reward attenuation [20,28].

Further, the retractable lever used in present study offered some distinct advantages. Motion of the lever gave a discriminate stimulus to the animals [35]. While the reward delivery was signaled by retraction of the lever, illumination provided the cue for the contingent activity [36]. This kind of arrangement generates good sign-tracking [37], discourages holding responses, and does not allow the animal to climb on the levers [35].

Modulation of the reward activity by CART peptides [intracerebroventricular (icv) or intra-pVTA route] or CART antibody (intra-pVTA route) was monitored (for detail procedure see Supplement 1). The schedule of treatment is summarized in Fig. 1.

2.3. Conditioning of the rats for CART self-administration

For intracranial self-administration (ICSA), we employed the procedure described by Rodd-Henricks et al. [6] with some modifications [38]. The pVTA cannulated rats were first trained to press lever for self-administration of sweet pellets and then allowed access to CART (55–102) for self-administration [39] (for details see Supplement 1). The infusion cannula was secured to the guide cannula, the animals placed individually in an operant chamber and were allowed to self-administer CART containing solution. On a reinforced response, a small cue light mounted above the active lever was illuminated during each 5-s infusion. Following each active lever press, a programmable microinfusion pump (E73-02; Coulbourn Instruments) connected to the apparatus delivered 100 nl of solution; the inactive lever press was ineffective. The infu-