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POSTERIOR HYPOTHALAMIC NUCLEUS DEEP BRAIN STIMULATION RESTORES LOCOMOTION IN RATS WITH HALOPERIDOL-INDUCED AKINESIA BUT NOT SKILLED FORELIMB USE IN PELLET REACHING AND LEVER PRESSING

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Abstract—Recent studies have shown that electrical stimulation of the posterior hypothalamic nucleus (PH) facilitates locomotion in control rats, and rats were made akinetic by dopaminergic blockade via haloperidol or dopamine depletion by the neurotoxin 6-hydroxydopamine. These findings suggest that PH stimulation might be a promising treatment for akinesia associated with dopamine loss in Parkinson's disease. The present study further examined the positive effects of PH stimulation on behavior by characterizing its potential facilitatory effects on tasks that require skilled movements. Rats were trained to reach for food pellets with a forelimb (skilled reaching) or press a bar in an operant conditioning task for food. PH stimulation in undrugged rats not only facilitated locomotion in each of the tasks, but also impaired performance of the skilled movement components of the tasks. Haloperidol reduced locomotion and skilled movement, and PH stimulation only restored locomotion. The results are discussed in relation to the idea that PH stimulation selectively facilitates locomotor behavior and may have limited use in restoring impairments in skilled movements and consummatory behavior that results from dopaminergic depletion.

Key words: Parkinson's disease, deep brain stimulation, posterior hypothalamic nucleus, akinesia, haloperidol, hypothalamus.

Electrical or pharmacological stimulation of the posterior hypothalamic nucleus (PH) elicits locomotor behavior as well as other behavioral and physiological effects (Bland and Vanderwolf, 1972; DiMicco et al., 1986; DiMicco and Abshire, 1987; Shekhar and DiMicco, 1987; Waldrop et al., 1988; Wible et al., 1988; Marciello and Sinnamon, 1990; Sinnamon et al., 1991; Sławińska and Kasicki, 1995, 1998; Oddie et al., 1996). Electrically elicited locomotion has been characterized as spontaneous and non-stereotypical behavior because an animal's movements can resemble spontaneous exploration and are integrated to its environment (Bland and Vanderwolf, 1972). There are many reciprocal and direct connections of the PH to other areas of the motor system including the motor cortex and thalamus, the cerebellum and diencephalic, subthalamic and reticulopontine locomotor regions, and the spinal cord that may facilitate such integrated behavior (Vertes et al., 1995; Vertes and Crane, 1996; Abrahamson and Moore, 2001; Cavdar et al., 2001a,b; Onat and Cavdar, 2003).

A potential therapeutic use of PH stimulation is in treatment of akinesia that characterizes Parkinson's disease (PD). In animal models of human PD in which akinesia is produced either pharmacologically by blocking dopamine (DA) receptors with haloperidol or by depleting DA bilaterally using the neurotoxin 6-hydroxydopamine, PH stimulation produces locomotion and in addition reinstates learned active avoidance that is abolished by DA blockade or depletion (Jackson et al., 2008; Young et al., 2009). These findings raise the question of the generality of the putative positive effects of PH stimulation in reversing the behavioral deficits of PD. For example, both humans with PD and animal models of PD are also impaired in making skilled movements with a forelimb (Whishaw et al., 2002). Particularly, they are impaired in the everyday activity of using a forelimb to reach for food and place it in the mouth for eating. A number of studies using animal models of PD (Metz et al., 2001) and studies of human PD patients suggest that skilled movements are more resistant to beneficial effects of contemporary therapies that successfully reduce akinesia (Melvin et al., 2005). The dissociation between locomotor and skilled movements with respect to therapy raises the question of whether the positive effects of PH might be generalized to skilled movements.

To examine the effects of PH stimulation on skilled movements, rats were trained on the single pellet reaching task, in which they reached for a food pellet with a forelimb (Whishaw et al., 2008), and on a bar pressing task using operant conditioning with fixed-ratio schedule. Haloperidol was also administered in pellet reaching and operant conditioning tasks to induce akinesia for examining the performance changes in the presence of PH stimulation. Additionally, the relationship between stimulation electrode placement in the PH region and the type of behaviors they elicited were mapped from implanted rats to provide clues of most effective PH stimulation sites that induce locomotion rather than unwanted effects.

EXPERIMENTAL PROCEDURES

Surgical procedures

Twenty-four male Long-Evans rats (350–450 g) were anaesthetized with ketamine/xylazine (100 mg/kg, 85:15 mix) for stereotaxic surgery. A bipolar stimulating electrode (Plastics One, MS303 twisted stainless steel) with tip separation of ~0.5 mm was

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Abbreviations: DA, dopamine; FR4, fixed ratio 4; PD, Parkinson's disease; PH, posterior hypothalamic nucleus.

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implanted in the PH (AP: −3.8, ML: 0.2, DV: 7.8 mm from dura). In addition, eight rats also received bipolar electrode implants to the hippocampus, motor cortex, and the dorsomedial striatum of which the data are not reported here. An additional untwisted bipolar electrode with insulation removed and tied onto a jeweller's screw was used as ground. Five additional jeweller's screws were fixed on the skull to serve as anchors for stabilizing the electrode implants along with dental cement. The rats received sutures and topical analgesia (Xylocaine, 2%, AstraZeneca, Mississauga, ON, Canada) upon the completion of surgery and were housed singly with food and water provided *ad libitum*. All rats were obtained from the Animal Care Facility at the University of Calgary and procedures adhered to the Canadian Council for Animal Care guidelines.

Qualitative behavioral testing

The rats were handled and acclimatized to the behavioral testing room after at least 7 days of recovery time post surgery. Once acclimatized, rats were connected to a Grass stimulator with a stimulus isolator and placed into the testing apparatus ($50\times40\times30$ cm^3). Using 0.1 ms monophasic pulses at 100 Hz, the stimulation intensity was gradually increased in 10 μA steps from 50 μA, and in 20 μA steps when the stimulation intensity was beyond 100 μA until a behavioral effect was observed. Typically, stimulation frequency $<$25 Hz did not elicit any observable effects. Increased whisking and postural shifts may be seen with stimulation frequency at around 50 Hz. Locomotion and other robust behavioral effects are only seen reliably with stimulation frequency $>$70 Hz. Usually, increased respiration and whisking can be observed immediately prior to any motor changes, which follow within 3 s of stimulation. The behavior of the rats was then recorded while continuous stimulation remained on for five or more minutes. Four distinctive types of motor behaviors were observed: (1) exploration-like locomotion; (2) unilateral head tilt or rotation; (3) transient (1–3 s) rhythmic, forelimb tic; and (4) flight-like locomotion. For exploration-like locomotion, the rats appear to be hyperactive but otherwise were able to engage in behaviors such as grooming and remain immobile for short periods (see supplementary Video 1 for an example). The rats never or very rarely tried to jump out of the testing apparatus, instead were exploring their surroundings with much rearing activity along the walls. Some rats displayed a unilateral preference in addition to increased locomotion. Generally, a unilateral head tilt appears initially, with full-blown rotational behavior with increasing stimulation intensity. A subset of the rats displayed abnormal motor tics, characterized by rhythmic forelimb flexion at the shoulder joint. The tic itself somewhat resembles seizure activity as the rats begin by rearing on their hind limbs and forced shift of posture by leaning toward one direction while ~5 Hz motor tic occurs on the side contralateral to the postural shift. However, the tic rate would decrease and eventually disappear within a few seconds of stimulation. After that, the rats may appear to behave normally or maintain a postural preference toward the side of the original postural shift during movement or immobility. Finally, flight-like locomotion is characterized by stimulus-bound sudden, ballistic locomotion from one side of the testing apparatus to the other. At the walls, the rats will always rear and frequently attempt to jump out of the testing apparatus. Some rats displaying this behavior also vocalized as they ran across the testing apparatus, or hanging from the top edge of the testing apparatus wall with attempts to jump out (see supplementary Video 2 for an example).

Pellet reaching

Training. Eight rats were food deprived at 90% of their free-feeding weight and trained in the pellet reaching task for 2 weeks (Whishaw et al., 2008). After the initial training, PH stimulating electrodes were implanted followed by a full week of recovery with food and water *ad libitum*, then a week of food deprivation with retraining. During the initial training phase, the rats were acclimatized in the clear Plexiglas apparatus ($45\times14\times35$ cm) with food pellets sprinkled within. At the same time, food pellets were available on a pedestal (2 cm above the apparatus floor) through a thin slit (1 cm wide) at the "front" of the box. As the rats began to reach, the preferred reaching paw was determined and pellet placement on the pedestal was adjusted accordingly to facilitate reaching. Also, the rats were shaped to travel to the back of the apparatus after successful or unsuccessful reaches by abstaining pellet placement until they did so. Therefore, each trial consisted of the rat reaching for the pellet, traveling to the back of the apparatus, and approaching the pedestal to reach for another pellet. All animals were trained to complete 20 trials daily, regardless of reach success. The same procedures were used during retraining after surgery.

Behavioral test. On test day, the rats received five practice trials before an injection of saline (0.9% NaCl, i.p.) was administered. Fifteen minutes after the injection, five trials with PH stimulation and five without were administered with the order of: off, on, on, off, off, on, on, off, off, on. Then, another injection of haloperidol (1 mg/kg, i.p.) was administered, and 15 min after the injection, the same testing protocol described for after saline injection was repeated. The number of trials completed (shuttling from pedestal to back of the apparatus and back again), the number of reaches attempted, and number of rears from saline and haloperidol sessions were recorded.

Operant conditioning

Training. Eight rats were used for this experiment. The original training consisted of a few days of acclimatization for handling, food neophobia, and the operant chamber. The rats were then shaped and trained on different reinforcement schedules for a different study that included fixed ratio and variable ratio reinforcement schedules. Upon the termination of the unrelated study, any contact with the operant chamber was ceased for a period of four weeks before the rats were retrained to receive a banana pellet for every four lever presses (FR4). Once all rats were receiving at least 80 pellets within 5 min, a single PH stimulating electrode was implanted followed by 5 days of recovery period. After recovery, the rats were again trained to criterion (80 pellets/5 min) on FR4 before testing began.

Behavioral test. Prior to the testing session, the rats were given about 1 min in the operant chamber with FR4 schedule before their removal and an injection of saline (i.p.) matching the volume of haloperidol injected later in the session. Each trial consisted of 2 min without PH stimulation and 2 min with stimulation, followed by haloperidol injection (1 mg/kg, i.p.) and a 15 min break for the drug to take effect. Another 2 min each with and without PH stimulation in the operant chamber was administered before the completion of the testing session. The rats were divided into two groups of four to counterbalance the order of stimulation. The frequency of lever presses and nose pokes into the food hopper were recorded as an indication of task performance.

Histology

At the completion of the experiments, the rats were given a lethal dose of sodium pentobarbital in preparation for transcardial perfusion. A saline (0.9% NaCl) injection to the left ventricle was followed by 10% formalin in saline. The brains were extracted and stored in 10% formalin and cryoprotected with 30% sucrose and 10% formalin mix prior to sectioning. Fifty μm slices from regions of interest were made in the cryostat, and electrode tracks were examined under a light microscope and digitally stored for later analysis.