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Operantly conditioned plasticity and circadian rhythm in rat H-reflex are independent phenomena

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Abstract

Recent studies indicate that rats can increase or decrease H-reflex amplitude in response to an operant conditioning paradigm. In addition, rats also display a circadian rhythm in H-reflex amplitude. As part of the development of H-reflex conditioning in the rat as a new model for defining the plasticity underlying a simple form of learning, this study examined the relationship in the rat between operantly conditioned H-reflex change and the H-reflex circadian rhythm. When H-reflex amplitude increased or decreased in response to the operant conditioning program, its circadian rhythm showed no changes in phase and minimal change in amplitude. Furthermore, animals did not alter daily performance schedule so as to use the rhythm to increase reward probability. Thus, in the rat, H-reflex operant conditioning and the H-reflex circadian rhythm appear to be independent phenomena. The circadian rhythm should not be a significant complicating factor in studies of operantly conditioned H-reflex change.

Keywords: H-Reflex; Circadian rhythm; Operant conditioning; Learning; Memory; Rats

A recent study demonstrated that rats can increase or decrease soleus H-reflex amplitude in response to an operant conditioning task [5]. This adaptive change occurs gradually over weeks. Animals can increase H-reflex amplitude more than 50% or decrease it more than 30% without change in background EMG or M response amplitude. The H-reflex, the simplest behavior of the vertebrate CNS, is produced primarily by the monosynaptic pathway consisting of the Ia afferent, the α-motoneuron, and the synapse between them. Thus, operant conditioning of the H-reflex in rats could provide a useful model for defining the plasticity underlying an adaptive change in vertebrate behavior and the learning processes responsible for it. A rat model would have significant advantages over the current primate H-reflex conditioning model [8–10], because it would greatly increase the range of possible experiments and the numbers of animals in each. Before this potential can be realized, the major features of H-reflex conditioning in the rat must be defined. Among these features is the relationship between operantly conditioned H-reflex change and the normal circadian variation in H-reflex amplitude.

The rat H-reflex displays a circadian rhythm that is opposite in phase to that found in the monkey [4,6,12]. In rats exposed to a normal light-dark cycle (lights bright 0600–2100 h, lights dim 2100–0600 h), H-reflex amplitude is larger around noon than around midnight, without change in background EMG or M response amplitude. The purpose of the present study was to determine whether conditioning of the H-reflex affected the phase or amplitude of the circadian rhythm and whether rats used the rhythm to increase reward probability (e.g. by performing more trials around noon when being rewarded for larger H-reflexes).

Animal preparation and data collection techniques are described in detail elsewhere [4,5]. They are summarized here. All procedures were in accord with DHEW Publ. No. (NIH) 85-23, 'Guide for the Care and Use of Laboratory Animals,' and had been reviewed and approved by the Institutional Animal Care and Use Committee of the Wadsworth Center. Male Sprague-Dawley rats (300–

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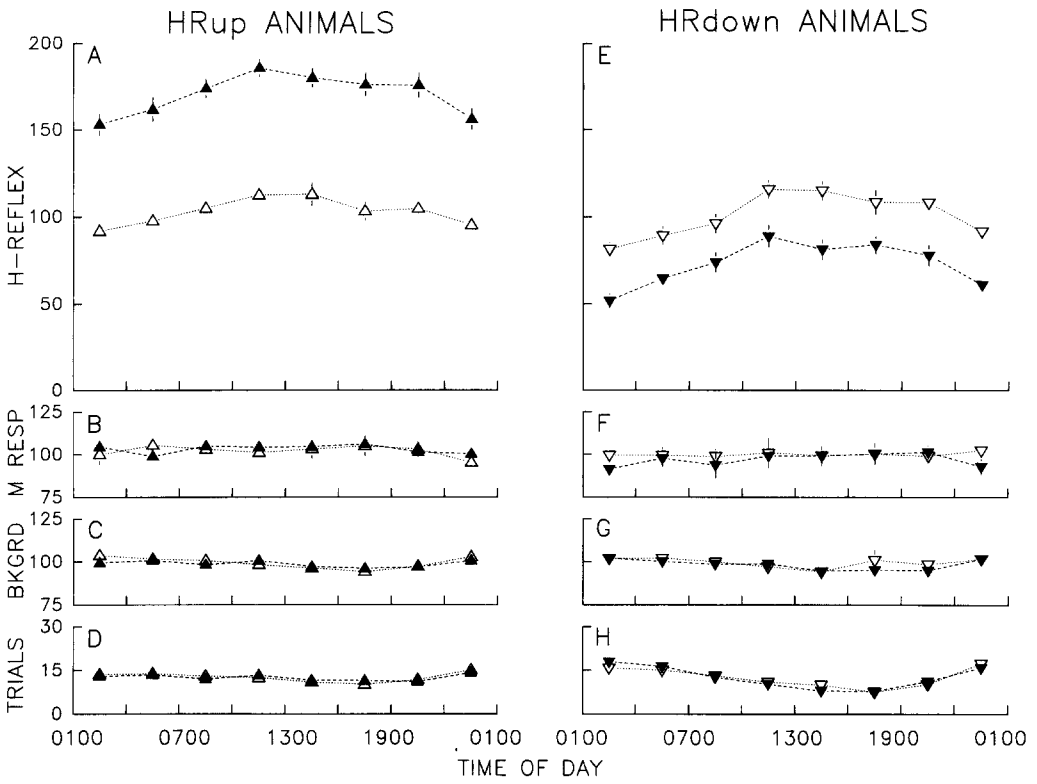

Fig. 1. Average values (±SE) of H-reflex amplitude, M response, background EMG, and number of trials (as percent of day's trials), for each 3-h period of the day for all HRup animals (A–D) and HRdown animals (E–H) under the control mode (dotted line and open triangles) and for the last 10 days of HRup mode exposure (dashed line and filled triangles). (Because H-reflex change under the HRup or HRdown mode varied widely across animals and the goal was to evaluate the circadian rhythm, prior to calculation of the means (±SE) presented here each animal's 3-h values after conditioning were divided by the animal's average H-reflex amplitude after conditioning and multiplied by the average for all animals after conditioning.)

500 g) were implanted under sodium pentobarbital anesthesia (60 mg/kg, i.p.) with chronic stimulating and recording electrodes in the right leg. To elicit the H-reflex, a nerve cuff containing fine-wire electrodes was placed around the right posterior tibial nerve just proximal to the triceps surae branches. To record bipolar soleus EMG activity, two fine-wire electrodes were placed in the soleus muscle. The Teflon-coated wires from the nerve cuff and from the muscle passed subcutaneously to a connector plug mounted on the skull. The reward used for operant conditioning was either a food pellet or intracranial stimulation. In rats for which reward was to be intracranial stimulation (i.e. ICS animals), a bipolar intracranial electrode (0.5 mm in diameter) was inserted stereotactically in left median forebrain bundle (MFB) at the level of the lateral hypothalamus (LH) and attached to the connector plug.

About 10 days after surgery, a 40-cm flexible cable was attached to the plug mounted on the skull. It conveyed the wires to an electronic swivel mounted above a standard rat cage, from which they passed to an EMG amplifier, a nerve-cuff stimulation unit, and, in the case of ICS animal, an ICS unit. The thin flexible cable allowed the animal to move freely about the cage and remained in place 24 h/day throughout 40–128 days of data collection. All animals had free access to water throughout data collection. ICS animals also had free access to standard rat chow throughout. During H-reflex conditioning, food animals received food mainly by performing the task described below. Lab lights were bright during the day (0600–21.00 h) and dim during the night (2100–06.00 h). Room temperature was 20°C. The weight and well-being of the animals were monitored carefully, and all remained healthy and active throughout data collection.

A minicomputer system digitized soleus EMG continuously, 24 h/day. When the EMG absolute value (equivalent to the full-wave rectified value) remained within a specified range for a randomly varying 2.3–2.7 s period, a brief stimulus pulse just above M response threshold was delivered by the nerve cuff. It elicited a threshold M response and an H-reflex. Under the control mode, the computer simply digitized soleus EMG and saved its absolute value for 50 ms following the stimulus. Under the conditioning mode (HRup or HRdown), the computer also provided a reward (food pellet or ICS) 200 ms after nerve stimulation if EMG amplitude in the H-reflex interval (typically 6–10 ms after nerve stimulation) was above (HRup mode) or below (HRdown mode) a criterion value. In the course of its normal daily activity, each rat satisfied the background EMG requirement and received nerve cuff

stimulation 2500–10 000 times per day. The computer provided data summaries for every 3-h and every 24-h period. Each summary included the number of trials, average level of background (i.e. prestimulus) EMG, average course of EMG amplitude for the 50-ms post-stimulus period, and (if in the HRup or HRdown mode) number of rewards. H-reflex amplitude was defined as average EMG amplitude in the H-reflex interval minus average background EMG amplitude and was expressed in units of background EMG amplitude.

Data were collected from each animal over 40–80 days. To determine initial H-reflex amplitude, the animal worked under the control mode for the first 3–10 days. Then it worked under the HRup or HRdown mode for 30–70 days. The background EMG criterion and the computer control of stimulus intensity [9] ensured that background EMG and M response amplitude normally remained stable within each day and throughout the 40–80 days of data collection. The data from those 3-h periods in which background EMG was not within 10% of its daily average and/or M response amplitude was not within 25% of its daily average were excluded from analysis [4]. H-reflex amplitude, M response, and background EMG were expressed as percents of average control mode values. Number of trials were expressed as percent of daily total. To determine the final effect of HRup or HRdown mode exposure on H-reflex amplitude, average H-reflex amplitude for the last 10 days of exposure was calculated as percent of initial (i.e. control mode) H-reflex amplitude.

The circadian rhythm data presented here are from 15 successfully conditioned rats (8 food and 7 ICS). A full description of the effects of conditioning in these animals was presented in an earlier paper [5]. After collection of control mode data, 9 were exposed to the HRup mode and 6 to the HRdown mode. H-reflex amplitude rose (HRup animals) or fell (HRdown animals) over several weeks, while background EMG and M response remained stable throughout. Figs. 1A–C show average values (±SE) for H-reflex, M response, and background EMG for each 3-h period of the day for the 9 HRup animals under the control mode (dotted line and open triangles) and for the last 10 days of HRup mode exposure (dashed line and filled triangles). After HRup mode conditioning, H-reflex amplitude averages 172% (±50% SD) of control, while M response and background EMG are unchanged. The circadian rhythm is evident under both control and HRup modes. Exposure to the HRup mode has no apparent effect on the phase of the H-reflex rhythm and minimal effect on its amplitude. Rhythm amplitude (measured as the average difference between the 6 h around noon and the 6 h around midnight and expressed in percent of the average amplitude for the eight 3-h periods in the day) is 13% (±8% SD) under the control mode and 16% (±15% SD) at the end of exposure to the HRup mode. No rhythm is evident in M response or background EMG amplitude.

Figs. 1E–G show comparable data from animals exposed to the HRdown mode. After HRdown conditioning, H-reflex amplitude averages 69% (±10% SD) of control, while M response and background EMG are unchanged. The circadian rhythm is comparable in phase under control and HRdown modes. Rhythm amplitude (measured as indicated above) was 29% (±16% SD) under the control mode and 38% (±24% SD) at the end of HRdown exposure. No rhythm is seen in background EMG or M response amplitude.

Figs. 1D and H show for HRup animals and HRdown animals the average number of trials (as percent of day's trials) for each 3-h period throughout the day under the control mode (dotted line and open triangles) and under the HRup or HRdown mode (dashed line and filled triangles). The number of trials performed in each 3-h period of the day is quite stable, though it is slightly lower in the afternoon. H-reflex conditioning does not affect the distribution of trials within each day. Thus, animals did not change their work schedules when exposed to the HRup or HRdown conditioning mode.

Rats can gradually increase or decrease H-reflex amplitude to increase reward probability without change in motoneuron tone as measured by background EMG and without change in stimulus strength as measured by M response amplitude [5]. The occurrence and direction of H-reflex change is wholly dependent on the reward contingency (i.e. HRup or HRdown), and thus appears to be a specific adaptive response to a specific external condition. The existence of a circadian rhythm in the H-reflex amplitude of the rat soleus muscle is not surprising since circadian rhythms are present in many aspects of CNS function.

The first significant finding of this study is that operantly conditioned change in rat H-reflex amplitude does not affect the phase of the circadian rhythm and has little effect on its amplitude. This result is consistent with a previous study showing that operantly conditioned change in the size of the primate biceps spinal stretch reflex (SSR) did not affect the circadian rhythm seen in that reflex [11]. The SSR and its electrical analog, the H-reflex, are the simplest behaviors of the vertebrate CNS [1,7]. Thus, the mechanisms underlying operantly conditioned change and the circadian rhythm must act at one or several of a small number of spinal sites. HRdown conditioning appears to involve change in motoneuron firing threshold and probably in Ia EPSP amplitude [2], while the mechanism of HRup conditioning remains unclear [3]. The minimal interaction between these operantly conditioned changes and the circadian rhythm suggests that the rhythm may have a different mechanism.

The second significant finding is that rats do not use the circadian rhythm to increase reward probability. Thus, those exposed to the HRup mode did not begin to perform more trials around noon when the H-reflex is normally larger, and those exposed to the HRdown mode did not

perform more trials around midnight when the H-reflex is normally smaller. Previous studies showed that monkeys also did not use the rhythm to increase reward probability [11]. This finding rules out a potential artifactual contribution to conditioned H-reflex change and thereby simplifies the design and interpretation of studies using this new model to study the plasticity underlying a specific form of learning.

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