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RAPID PUBLICATION

Operant Conditioning of H-reflex in Freely Moving Rats

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SUMMARY AND CONCLUSIONS

1. Primates can increase or decrease the spinal stretch reflex and its electrical analogue, the H-reflex (HR), in response to an operant conditioning task. This conditioning changes the spinal cord itself and thereby provides an experimental model for defining the processes and substrates of a learned change in behavior. Because the phenomenon has been demonstrated only in primates, its generality and theoretical implications remain unclear, and its experimental use is restricted by the difficulties of primate research. In response to these issues, the present study explored operant conditioning of the H-reflex in the rat.

2. Seventeen Sprague-Dawley rats implanted with chronic electromyographic (EMG) recording electrodes in one soleus muscle and nerve cuff stimulating electrodes on the posterior tibial nerve were rewarded (either with medial forebrain bundle stimulation or food) for increasing (HRup conditioning mode) or decreasing (HRdown conditioning mode) soleus H-reflex amplitude without change in background EMG or M response (direct muscle response) amplitude.

3. H-reflex amplitude changed appropriately over 3–4 wk. Under the HRup mode, it rose to an average of 158 ± 54% (mean ± SD) of initial value, whereas under the HRdown mode it fell to an average of 67 ± 11% of initial value. Background EMG and M response amplitude did not change.

4. Operant conditioning of the H-reflex in the rat appears similar in rate and final magnitude of change to that observed in the monkey. Additional studies are needed to determine whether it displays comparable muscular specificity, follows a similar two-phase course, and is also accompanied by spinal cord modifications.

5. The results indicate that adaptive plasticity of the behavior mediated by the spinal stretch reflex pathway can occur in a subprimate species. With further refinement of the experimental protocol and careful attention to the inherent variability of the rat H-reflex, operant conditioning of the H-reflex in the rat should provide a useful model for defining the plasticity underlying an adaptive change in vertebrate behavior and the learning processes responsible for it.

INTRODUCTION

The spinal stretch reflex (SSR), the earliest response to sudden muscle stretch, is the simplest behavior of the vertebrate CNS. It is mediated by a wholly spinal and largely monosynaptic pathway, consisting of the primary afferent neuron, the α-motoneuron, and the synapse between them (Brown 1984; Matthews 1972). Both monkeys and humans can increase or decrease the SSR or its electrical analogue, the H-reflex, when exposed to an operant conditioning paradigm in which reward depends on reflex amplitude (Evatt et al. 1989; Wolpaw 1987; Wolpaw et al. 1983a; for review, see Wolpaw and Carp 1990). Change begins quickly and progresses steadily over days and weeks. Most important, the conditioned reflex asymmetry survives the removal of supraspinal influence (Wolpaw and Lee 1989). Thus part of the CNS plasticity responsible for this learned change in behavior occurs in the spinal cord, where it constitutes a potentially accessible memory trace. Recent intracellular studies in conditioned animals indicate that this trace involves the motoneurons themselves as well as other spinal cord sites (Carp and Wolpaw 1994).

Up to the present, operant conditioning of the SSR pathway has been demonstrated and explored only in the primate CNS. As a result, the generality and theoretical implications of the phenomenon remain undefined. Furthermore, the present restriction to primates limits the use of this new experimental model in exploring the processes of learning and the substrates of memory. Demonstration of the phenomenon in an appropriate subprimate species would both clarify its theoretical implications and increase its practical importance as an experimental model. In the study reported here, we set out to develop an H-reflex operant conditioning paradigm for the laboratory rat, which is becoming the model of choice for many studies of mammalian CNS function in general and spinal cord function in particular. Preliminary results have appeared in abstract form (Chen and Wolpaw 1993, 1994b).

METHODS

Experimental design and techniques were adapted from those used for operant conditioning of the H-reflex in freely moving monkeys (Wolpaw and Herchenroder 1990). The main difference was that the reward in the present study was either food pellet or intracranial stimulation, rather than fluid. All animal procedures were in accord with Department of Health, Education, and Welfare (DHEW) Publication No. (National Institutes of Health) 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.

Sprague-Dawley rats (male, 300–500 g) were implanted under pentobarbital sodium anesthesia (60 mg/kg ip) with chronic stimulating and recording electrodes in the right leg. To elicit the H-reflex, a silicone rubber nerve cuff containing a pair of stainless steel multistranded fine-wire electrodes was placed around the posterior tibial nerve just proximal to the triceps surae branches. To record bipolar soleus electromyographic (EMG) activity, two fine-wire EMG electrodes with their final 0.5-cm segments stripped and separated by 0.2–0.3 cm were placed in the soleus muscle. The Teflon-coated wires from the nerve cuff and the muscle passed subcutaneously to a connector plug mounted on the skull. In the rats for which reward was to be intracranial stimulation (i.e., ICS animals), the connector also held a bipolar stimulating electrode that was placed stereotaxically in the left medial forebrain bundle (MFB) at the level of the lateral hypothalamus. Seven to 10 days

after surgery, ICS rats were tested in a bar press chamber to ensure that ICS was rewarding and to determine the amount of 60-Hz current required (usually 10–20 μA for 200–300 ms). All rats were tested with nerve-cuff stimulation to ensure that an H-reflex was present at M response (i.e., direct muscle response) threshold.

Data were gathered from 17 rats (10 food and 7 ICS) for at least 40 days. Throughout this period, each animal lived in a standard rat cage. A 40-cm flexible cable was attached to the plug mounted on the skull. It carried the wires to an electronic swivel above the cage and thence to an EMG amplifier (1,000 gain, 100- to 5,000-Hz bandpass), a nerve-cuff stimulation unit, and, in the case of ICS animals, an intracranial stimulation unit. The cable, which allowed the animal to move freely about its cage, remained in place 24 h/day. All animals had free access to water and standard rat chow, except that, during H-reflex conditioning, food animals received food mainly by performing the task described below. Animal well-being was carefully monitored. Body weight was assessed every 2 weeks for ICS animals and every week for food animals. Lab lighting was dimmed from 2100 to 0600 each day.

A minicomputer system interfaced with up to four rats 24 h/day. For each animal, it monitored soleus EMG and controlled two outputs: the nerve-cuff stimulus and reward (i.e., either ICS stimulation or 20-mg food pellet). If the absolute value (equivalent to the full-wave rectified value) of background (i.e., ongoing) EMG remained within a specified range (usually 1–2% of maximum possible EMG, which was assessed as maximum M response) for a randomly varying 2.3- to 2.7-s period, a stimulus pulse (typically 0.5 ms in duration) was delivered by the nerve cuff. Pulse amplitude was kept at M response threshold (Wolpaw and Herchenroder 1990). The M response began ~1.5 ms after nerve stimulation and lasted ~3 ms. The H-reflex started at ~6 ms and lasted ~4 ms. Under the control mode, the computer simply digitized soleus EMG and stored its absolute value for 50 ms after the stimulus. Under the HRup or HRdown conditioning mode, it gave 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 activity, each animal usually satisfied the background EMG requirement and thus received nerve-cuff stimulation 2,500–10,000 times per day. Trials were distributed throughout the 24 h of each day but tended to be less frequent in the afternoon (Chen and Wolpaw 1994a). H-reflex amplitude was calculated as average EMG amplitude in the H-reflex interval minus average background EMG amplitude and was expressed in units of background EMG amplitude. H-reflex amplitude varied across animals, ranging from 0.3 to 1.7 times background EMG amplitude.

For each rat, data were collected under the control mode for 3–10 days. These data defined the animal's initial H-reflex amplitude. Then it was exposed for 30–70 days to the HRup (6 ICS and 5 food animals) or HRdown mode (1 ICS and 5 food animals). The reward criterion was normally set on the basis of the control mode data so that 10–40% of the trials were rewarded. As H-reflex amplitude changed appropriately over subsequent days and weeks, the criterion was changed so as to maintain reward probability in this range.

The background EMG criterion that had to be met before the stimulus was delivered ensured that daily background EMG was normally within 10% of its initial (i.e., control-mode) value throughout data collection. Those days that differed by >10% were excluded from analysis. The trial-to-trial computer control of stimulus strength (Wolpaw and Herchenroder 1990) ensured that M response amplitude did not increase or decrease across the prolonged period of data collection. At the same time, presumably because M response amplitudes typically display a skewed distribution and the computer controlled *median* M response amplitude, mean M response amplitude displayed substantial day-to-day variation. Those days for which mean M response amplitude differed by >15% from its initial (i.e., control-mode) value were excluded from analysis.

To determine the final effect of HRup or HRdown mode exposure on H-reflex amplitude, average H-reflex amplitude for the final 10 days of exposure was calculated as percent of initial (i.e., control-mode) H-reflex amplitude. As with operant conditioning in the monkey (Wolpaw et al. 1989), a change of ≥20% in the correct direction was considered evidence of successful training. In addition, each animal's H-reflex amplitudes for the final 10 days were compared with those of its control-mode days by *t*-test.

At the end of study, each animal was killed with an intraperitoneal overdose of pentobarbital sodium and perfused through the heart with saline followed by 4% formaldehyde solution. Muscle and nerve condition and electrode placement were examined. Right and left soleus muscles were weighed. For ICS animals, the brain was removed and examined to verify location of the ICS electrode in the left MFB.

RESULTS

Animals remained healthy and active throughout data collection. Body weight increased from 300–500 g at the time of implantation surgery to 550–750 g at the time of perfusion. Soleus muscle weights (measured as percent of body weight) were symmetrical and did not differ significantly between HRup and HRdown animals. Examination of the nerve cuffs revealed the expected connective tissue investment of the wires and apparent good preservation of the nerve inside the cuff. In ICS animals, a lesion 0.6–1.2 mm diam consistent with placement and use of the ICS electrode was found in the region of the medial forebrain bundle.

Figure 1 shows average daily H-reflex amplitude, M response amplitude, and background EMG for all HRup animals (▲) and HRdown animals (▼) during the control-mode period and during exposure to the HRup or HRdown mode. H-reflex amplitude rises (HRup animals) or falls (HRdown animals) over several weeks, whereas background EMG and M response remain stable throughout. In the 11 HRup animals, H-reflex amplitude rose to 158 ± 54% (mean ± SD) of its control-mode value. In 9 of the 11, H-reflex amplitude rose ≥20% and thus met the standard criterion for successful training. In the other two, it remained within 20% of control (i.e., 112 and 82% of control, respectively). In the six HRdown animals, H-reflex amplitude fell to 67 ± 11% of its control-mode value, and all six were successful by the standard criterion.

For each of the 15 successful animals, H-reflex amplitude for the final 10 days differed in the appropriate direction from the control-mode days by *t*-test ($P < 0.01$ for each of 14 animals, $P < 0.05$ for the remaining animal). For HRup conditioning, average final H-reflex amplitude was larger in the six ICS animals than in the five food animals (i.e., 173 ± 60% versus 139 ± 46%). However, the difference was not significant ($t = 1.02$, $P > 0.3$) and was ascribable to larger initial H-reflex amplitudes in these five food animals (see Wolpaw et al. 1993 for discussion of this factor). For HRdown conditioning, final H-reflex amplitude for the one ICS animal was 61%, close to the group mean.

Figure 2 shows the course and final effect of HRup (*left*) or HRdown (*right*) conditioning in two animals. In both animals, background EMG and M response amplitude (area under the curve) are comparable before and after condition-

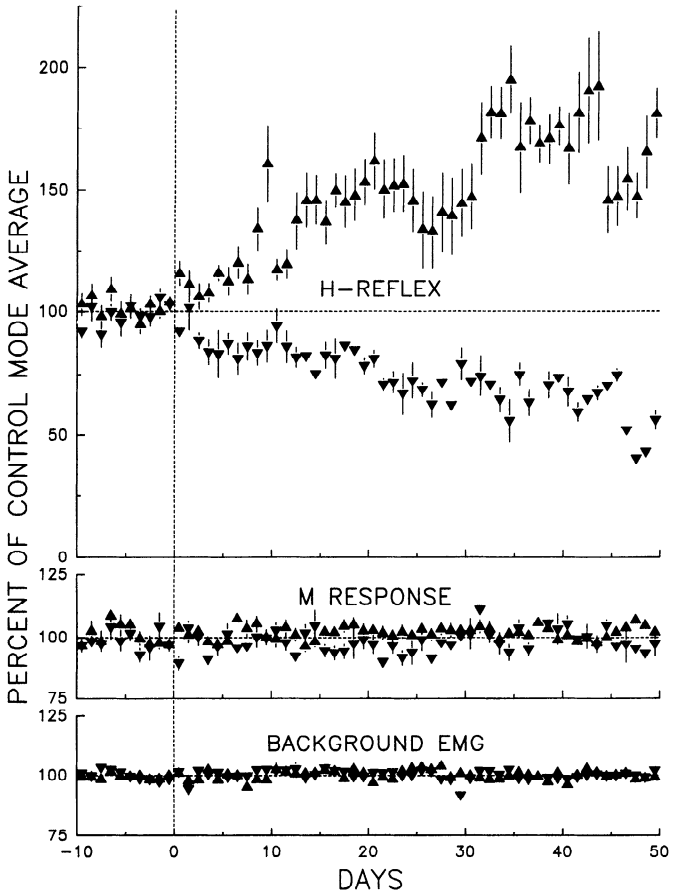

FIG. 1. Average daily H-reflex amplitude, M response amplitude, and background electromyographic (EMG) for all HRup animals (▲) and HRdown animals (▼) during the control mode and the period of exposure to the HRup or HRdown mode (in percent of control-mode value). Bars indicate standard errors. H-reflex amplitude rises (HRup animals) or falls (HRdown animals) over several weeks, whereas background EMG and M response remain stable throughout data collection.

ing. In contrast, H-reflex amplitude is much larger after HRup exposure and much smaller after HRdown exposure.

DISCUSSION

The results indicate that rats, like primates, can gradually increase or decrease H-reflex amplitude to increase reward probability without change in background motoneuron tone (as measured by background EMG) and without change in stimulus strength (as measured by M response amplitude). The occurrence and direction of H-reflex change depends on the reward contingency (i.e., HRup or HRdown) and thus appears to be a specific adaptive response to a specific external condition. Evidence from several sources indicates that adaptive change in the primate SSR pathway is not simply a laboratory phenomenon. It occurs during normal development and during acquisition of motor skills (Goode and Van Hoeven 1982; Meyer-Lohmann et al. 1986; Myklebust et al. 1986). The present results further establish the generality of the phenomenon by demonstrating that it occurs in a subprimate species in response to two very different reward modalities (i.e., food and ICS).

Soleus H-reflex conditioning in the rat appears similar in rate and magnitude to triceps surae H-reflex conditioning in the monkey (Wolpaw 1987; Wolpaw et al. 1993). Both species perform comparable numbers of trials per day. Although both display a spontaneous diurnal rhythm in reflex amplitude (Chen and Wolpaw 1994a; Dowman and Wolpaw 1989), neither adjusts its daily performance schedule so as to use the rhythm to increase reward probability (e.g., under the HRup mode, rats do not perform more trials in the afternoon, even though the H-reflex is usually larger at this time) (Wolpaw et al. 1984).

Additional studies are needed to determine whether H-reflex conditioning in the rat, like that in the primate, is focused on the muscle being conditioned, and whether it displays the two-phase course of change seen with primate SSRup, SSRdown, HRup, and probably HRdown conditioning (Wolpaw and O'Keefe 1984; Wolpaw et al. 1994). Most important, it remains to be determined whether H-reflex conditioning modifies the rat spinal cord and, if so, whether the modifications resemble those occurring in the monkey.

Because the H-reflex is produced primarily by the Ia afferent synapse on the α-motoneuron and the α-motoneuron itself, its amplitude depends on the state of these two CNS elements. Both are subject to influence exerted by descending pathways from supraspinal regions (Baldissera et al. 1981; Burke and Rudomin 1978). It is presumably mode-appropriate change in this supraspinal influence that is operantly conditioned by exposure to the HRup or HRdown mode. Because the H-reflex is elicited at an unpredictable time (see METHODS) and occurs before any other CNS response, the animal can only increase reward frequency by being prepared ahead of time, i.e., by continuously controlling the descending activity so as to maintain the spinal reflex arc in the proper state. As a result, mode-appropriate supraspinal influence is present for prolonged periods each day as the animal, rat or monkey, performs the task over the many days of HRup or HRdown exposure.

In monkeys, this long-term supraspinal influence over the spinal arc eventually produces activity-driven plastic changes in the spinal cord. This plasticity is manifested by reflex asymmetries that persist after removal of supraspinal influence (Wolpaw and Lee 1989). It appears to involve changes at several sites in the spinal cord (Carp and Wolpaw 1994; Wolpaw and Lee 1989). The use of equivalent experimental protocols and the observation of comparable effects on H-reflex amplitude suggest that H-reflex conditioning in the rat, like that in the monkey, changes the spinal cord. At the same time, the underlying neuronal mechanisms may be different in the two species. The direct corticomotoneuronal connections found in primates and thought to support fine motor control may be less prominent in the rat (Liang et al. 1991; Porter and Lemon 1993). As a result, the supraspinal influence responsible for operantly conditioned H-reflex change may be less precisely focused on the muscle controlling reward. Thus, soleus H-reflex conditioning in the rat might be accompanied by comparable change in other ipsilateral hindlimb muscles or even in contralateral hindlimb muscles, and thereby differ markedly from the relatively focused and unilateral change seen in the monkey (Wolpaw et al. 1983b, 1989, 1993).

If H-reflex conditioning does change the rat spinal cord, it should provide an excellent opportunity for defining the

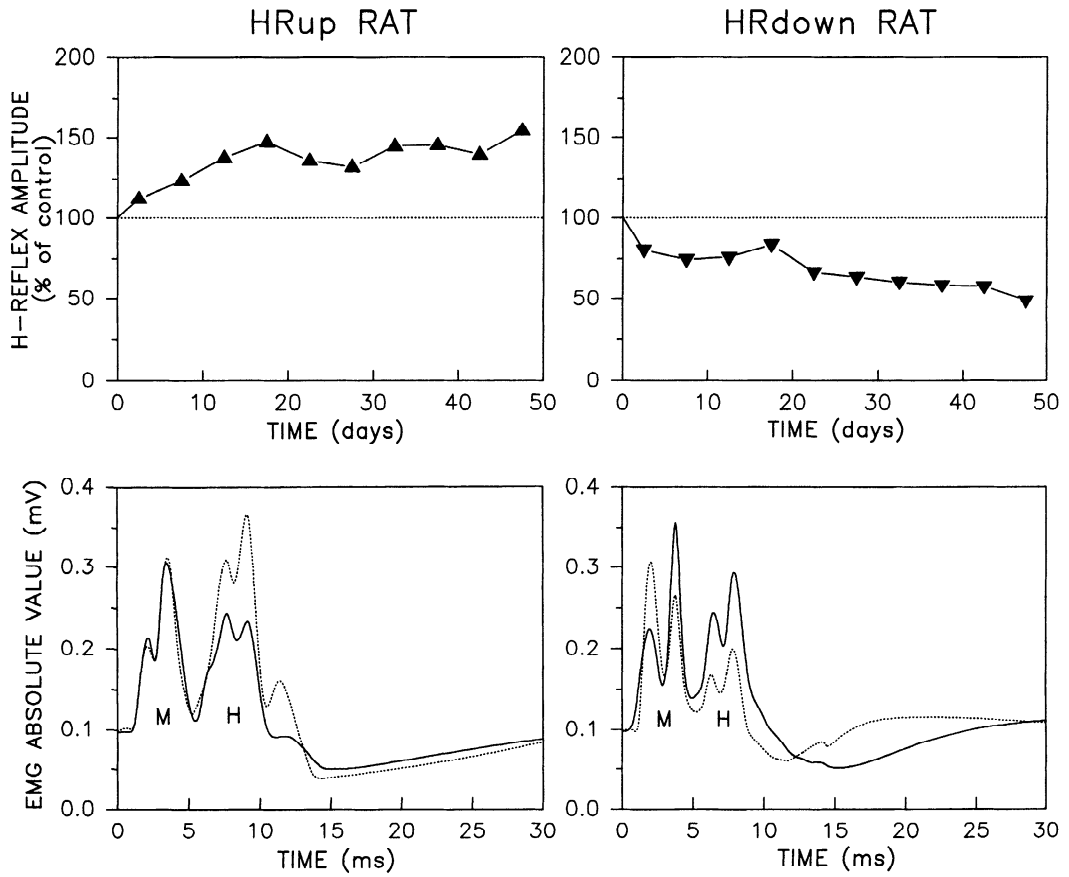

FIG. 2. Data from 1 HRup rat (*left*) and 1 HRdown rat (*right*). *Top*: average H-reflex amplitude for each 5-day period after onset of HRup or HRdown conditioning (in percent of control-mode H-reflex amplitude). *Bottom*: average poststimulus EMG response to the nerve-cuff stimulus for a day before (——) and a day after (· · ·) prolonged HRup or HRdown exposure. The H-reflex is much larger after HRup conditioning and much smaller after HRdown conditioning, whereas background (i.e., prestimulus) EMG and M response (i.e., area under the curve) do not change.

CNS plasticity underlying an operantly conditioned behavioral change and the learning processes that create this plasticity. Unlike the primate model, the use of which is limited by necessary restriction to small study populations, the rat model should permit a wide variety of detailed studies, such as efforts to manipulate pharmacologically and otherwise the events occurring during conditioning.

The previous studies of SSR and H-reflex conditioning in the monkey revealed no changes in posture or other visible behavior accompanying reflex conditioning. The long-term stability of M response amplitude and the fact that H-reflex change in the rat, like that in the monkey, occurs gradually makes mediation by a simple postural change less likely. Nevertheless, the occurrence of such associated phenomena in the rat and their potential role in conditioning requires exploration. These studies could employ additional EMG channels to monitor activity in closely related muscles (e.g., the antagonist tibialis anterior), as well as in more remote muscles.

Productive use of this new experimental model will require attention to the fact that H-reflex amplitude in the rat varies substantially from moment to moment and day to day, even when background EMG is controlled. Although some of this variation might be reduced by methodological improvements, most of it appears to be inherent in the phenomenon, a product of the multiple influences impinging on the motoneuron and its Ia afferent synapse, and must be taken into account in the planning and interpretation of experiments. Sufficient number of trials must be performed over sufficient periods of hours and days, and analyses of the effects of conditioning must be based on performance over periods of sufficient length.

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