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Neuroscience Letters 302 (2001) 85–88

Neuroscience Letters

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# Time course of H-reflex conditioning in the rat

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Received 13 November 2000; received in revised form 30 January 2001; accepted 21 February 2001

**Abstract**

This study sought to define the course of operantly conditioned change in the rat soleus H-reflex and to determine whether, like H-reflex conditioning and spinal stretch reflex conditioning in the monkey, it develops in distinct phases. Data from 33 rats in which the right soleus H-reflex was trained up (i.e. HRup mode) and 38 in which it was trained down (i.e. HRdown mode) were averaged to define the courses of H-reflex increase and decrease. In HRup rats, the H-reflex showed a large phase I increase within the first 2 days followed by gradual phase II increase that continued for weeks. In HRdown rats, the H-reflex appeared to show a small phase I decrease and then showed a gradual phase II decrease over weeks. In combination with other recent work, the data suggest that H-reflex conditioning begins with a rapid mode-appropriate alteration in corticospinal tract influence over the spinal arc of the H-reflex, which causes phase I change, and that the continuation of this altered influence induces gradual spinal cord plasticity that is responsible for phase II change. The results further establish the similarity of H-reflex conditioning in primates and rats. Thus, they encourage efforts to produce a single coherent model of the phenomenon based on data from the two species and indicate the potential clinical relevance of the rat data. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

*Keywords*: Conditioning; Rat; Plasticity; Learning; Memory; Reflex; Spinal cord

Operant conditioning of the spinal stretch reflex (SSR) or of its electrical analog, the H-reflex, has been described in monkeys, humans, and rats [5,9,12,13,15–18]. SSR or H-reflex change develops over weeks and is associated with persistent functional and structural changes in the spinal cord itself. This phenomenon provides an experimental model for exploring the plasticity underlying a learned change in behavior, and could also provide methods for guiding recovery of function after spinal cord injury.

In the monkey, both SSR and H-reflex conditioning appear to occur in two phases: a rapid phase I change that occurs in the first 1–2 days, and a slow phase II change of 1–2%/day that continues for many days [21,22]. Phase I change appears to reflect rapid operantly conditioned alteration in supraspinal influence over the spinal arc of the SSR or H-reflex, and presumably occurs because it causes rapid and substantial increase in reward probability. Phase II change is attributable to the gradual development of plastic changes in the spinal cord due to the continued presence of this altered supraspinal influence. Recent work in rats indicates that the main corticospinal tract is essential for H-reflex conditioning: H-reflex conditioning does not occur if this tract is transected [4,7]. This finding suggests that the supraspinal influence responsible for phase I change and phase II change is carried by the corticospinal tract. However, it is not known whether the two-phase change seen in monkeys also occurs in rats. The presence of two-phase change in the rat would further establish the similarity of H-reflex conditioning in the two species, and thus would support efforts to derive from primate and rat data a single coherent model of the H-reflex conditioning phenomenon. It would also indicate the potential relevance of the rat data to the development of new clinical methods.

The present study addresses the question of the time course of H-reflex change in the rat. It presents data from 71 rats (Sprague–Dawley, 36 females and 35 males, initial weight 203–646 g) that underwent successful conditioning of the soleus H-reflex (i.e. the H-reflex increased (HRup conditioning mode) or decreased (HRdown conditioning

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PII: S0304-3940(01)01658-5

mode) by ≥20% from its initial value [18,19][1]). All procedures satisfied the 'Guide for the Care and Use of Laboratory Animals' of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, D.C., 1996) and had been reviewed and approved by the Institutional Animal Care and Use Committee of the Wadsworth Center. The H-reflex conditioning protocol for rats, described in detail elsewhere [5], is summarized here.

Each rat was implanted under anesthesia with fine-wire electromyographic (EMG) electrodes in the right soleus muscle and nerve cuff stimulating electrodes on the posterior tibial nerve. Electrode wires passed subcutaneously to a head mount. Data collection started at least 10 days later. During collection, the rat lived in a standard rat cage with a 40 cm flexible tether connected to the head mount. The tether, which allowed the rat to move freely about the cage, conveyed the electrode connections to an electronic swivel above the cage and from there to an EMG amplifier and a nerve-cuff stimulation unit. The rat had continuous access to food and water, except that during H-reflex conditioning it received food primarily by performing the task described below. Animal well-being was checked carefully several times each day, and body weight was measured each week. Laboratory lights were reduced from 21:00 to 06:00 daily.

A computer system continuously monitored soleus EMG and controlled the nerve cuff stimulus. If the absolute value (i.e. equal to the full-wave rectified value) of background (i.e. ongoing) EMG stayed in a defined range for a randomly varying 2.3–2.7 s period, a stimulus pulse (normally 0.5 ms in duration) was given by the nerve cuff. Pulse amplitude was initially set just above M-response threshold and then automatically and continuously adjusted to keep M-response amplitude stable over the weeks of data collection. Under the control mode, the computer merely digitized (at ≥2,000 Hz) and stored the absolute value of soleus EMG for 50 ms after stimulation. Under the HRup or HRdown conditioning mode, it also gave a food reward 200 ms after stimulation if EMG amplitude in the H-reflex interval (typically 5.5–9.0 ms after stimulation) was greater than (HRup mode) or less than (HRdown mode) a criterion value. In the course of its normal activity, the rat fulfilled the background EMG requirement, and thus received nerve stimulation, 2000–10 000 times per day. Each rat was exposed to the control mode for at least 10 days and then to the HRup or HRdown mode for at least 40 days. (Except for 3 HRdown rats in which loss of the head mount or malfunction of the implanted electrodes ended data collection after 32 or 33 days of HRdown exposure.)

The computer gave a daily summary that included number of trials, number of rewards, average background EMG amplitude immediately prior to nerve stimulation, and the course of average EMG amplitude for 50 ms after stimulation. 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 average background EMG amplitude, which remained stable throughout data collection.

At the end of study, each rat received an overdose of sodium pentobarbital (i.p.) and was perfused through the heart with saline followed by 4% paraformaldehyde (or 3% paraformaldehyde and 1% glutaraldehyde) in 0.1 M phosphate buffer (pH 7.3). Placement of the EMG electrodes and the nerve cuff and the integrity of the tibial nerve were verified. The soleus muscles of both sides were removed and weighed.

Fig. 1 shows average daily H-reflex amplitudes (±SEM) (in percent of average amplitude for the final 10 days of control-mode exposure) in HRup and HRdown rats for the final 10 days of control-mode exposure and for days 1–40 after onset of the HRup or HRdown mode. The solid linear regression lines confirm the very high correlations between

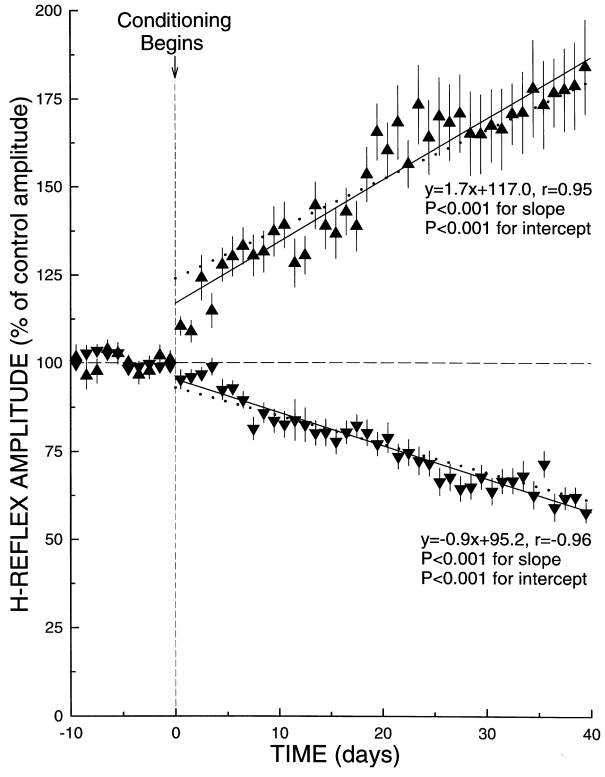


Fig. 1. Average daily H-reflex amplitudes (±SEM) as percent of initial (i.e. control-mode) amplitude in HRup and HRdown rats for 10 days before and 40 days after onset of the HRup(▲)or HRdown(▼) mode. The solid linear regression lines, with their parameter values and significances, are for the upper (rat HRup data) and lower (rat HRdown data) plots. The dotted linear regression lines are from an earlier study of the courses of HRup and HRdown conditioning in the triceps (TS) muscles of the monkey [21].

[1] These 71 rats were drawn from a larger group of 91. In the other 20 rats, conditioning was not successful, i.e. H-reflex amplitude remained within 20% of its initial value throughout 50 days of exposure to the HRup or HRdown mode.

Table 1
Comparison of phase I and phase II changes in the rat soleus H-reflex (present study), the monkey triceps surae (TS) H-reflex [21], and the monkey biceps spinal stretch reflex (SSR) [22][a]

| | Rat soleus H-reflex | Monkey TS H-reflex | Monkey biceps SSR |
|---|---|---|---|
| Up conditioning | | | |
| Phase I | 17.0% | 24.0% | 8.7% |
| Phase II | 1.7%/day | 1.4%/day | 1.2%/day |
| Down conditioning | | | |
| Phase I | −4.8% | −7.0% | −7.7% |
| Phase II | −0.9%/day | −0.8%/day | −0.8%/day |

[a] The phase II values for the rat and monkey H-reflexes are the slopes of the linear regressions for days 0–40, while the phase II values for the monkey SSR are average values calculated from the values of the fitted curves at 40 days minus the phase I values [22].

H-reflex amplitude and days of HRup or HRdown conditioning ($r = 0.95$ and $r = -0.96$, respectively). Furthermore, the y-intercepts of both regressions are significantly different from 100% ($P < 0.001$)[2]. The dotted linear regression lines are from an earlier study of HRup and HRdown conditioning in the monkey triceps surae (TS) muscles [21]. While phase I change is somewhat larger and phase II change slightly slower in the monkey, the courses of H-reflex conditioning in the two species are similar.

Fig. 1 is consistent with the conclusion that H-reflex increase in HRup rats (as in HRup monkeys) occurred in two phases: rapid phase I change of 17% (estimated from the *y*-intercept) over the first 1–2 days and slow phase II change of 1.7%/day (estimated from the slope) that continued through the rest of the 40 days. Phase II change was responsible for most of the final change. Fig. 1 also suggests that H-reflex decrease in HRdown rats occurred in two phases: a small phase I change of about −5% in the first 2 days and slow phase II change of −0.9%/day that continued for the remainder of the 40 days and was responsible for most of the final change.

The courses and final magnitudes of H-reflex increase and decrease in the rat soleus shown in Fig. 1 are similar to those previously described for TS H-reflex conditioning and biceps SSR conditioning in the monkey [21,22]. Table 1 compares the phase I and phase II changes found in the present study to those found in the earlier studies. For biceps SSR conditioning in the monkey, both SSRup and SSRdown conditioning show definite phase I and phase II changes, and the phase I changes are nearly equal in absolute value and clearly occur within 6 h [22]. In contrast, H-reflex conditioning of rat soleus and monkey TS both show a large phase I change for HRup conditioning and a relatively small phase I change for HRdown conditioning, and these phase I changes appear to occupy several days. As previously discussed [21], data on the behavior of the contralateral (i.e. unconditioned) H-reflex in the monkey suggest that the phase I asymmetry (i.e. absolute value of phase I greater for HRup conditioning than for HRdown conditioning) reflects a nonspecific rapid increase in H-reflex amplitude associated with onset of the reward contingency. It might be due to a Jendrassik maneuver, which could increase H-reflex amplitude without affecting background EMG level (probably by changing presynaptic inhibition at the Ia synapse [1,8,24]). This nonspecific increase probably adds to a mode-specific phase I increase in HRup animals and detracts from a mode-specific phase I decrease in HRdown animals. The alternative explanation for the asymmetry, that mode-specific phase I decrease is in fact very small or absent with HRdown conditioning, does not account for the behavior of the contralateral H-reflex in the monkey and makes it more difficult to understand H-reflex decrease as an operantly conditioned phenomenon [21]. The longer time occupied by phase I in the average H-reflex data from rats or monkeys (i.e. 1–2 days vs. 6 h for the monkey SSR data) is likely to reflect greater between-animal variation in the exact time of onset of phase I. This difference may be attributable to the fact that soleus (or triceps surae) H-reflex conditioning probably constitutes a more difficult learning problem than biceps SSR conditioning [21]. SSR conditioning involves a more physiological stimulus, a muscle that is probably under more direct supraspinal control, a response that has lower spontaneous variability, and a protocol that ensures that the muscle is essentially totally committed to the task. At the same time, for both up and down conditioning, phase II change is similar in rate in the three studies summarized in Table 1. Because phase II continues for at least 40 days, it is responsible for most of the final amplitude change.

Phase II appears to reflect the CNS plasticity responsible for the adaptive change in H-reflex amplitude, while phase I

[2] A polynomial regression of the form $y = a + bx + cx^2$ (where $x$ is days, $y$ is H-reflex amplitude, and a, b, and c are free parameters) did not account for substantially more of the variance than did the linear regressions (i.e. $r$ for the HRup data went from 0.95 to 0.96 and $r$ for the HRdown data went from −0.96 to −0.97). For the HRup data, the intercept and the linear and quadratic trends were significant ($P < 0.01$, 0.01, 0.05, respectively). The significant intercept indicates that this regression is consistent with the phasel/phase II model for HRup conditioning. For the HRdown data, the intercept did not reach significance, while the linear and quadratic trends were significant ($P = 0.15$, $P < 0.01$, $P < 0.02$, respectively). As noted below, HRdown phase I change is probably obscured by a nonspecific factor associated with the onset of conditioning.

reflects the mechanisms that create it. In terms of the behavioral change (i.e. a larger or smaller H-reflex), phase II represents the memory trace that underlies the change and phase I represents the learning process that creates the trace. That phase II reflects spinal cord plasticity is suggested by its slow development, reversal, and redevelopment [6,14,17] and by its persistence [23], and has been documented by the demonstration that conditioned reflex change remains in the spinal cord after complete transection [20]. Physiological and anatomical studies indicate that phase II involves plasticity at multiple sites in the spinal cord. Changes specific to the conditioning mode (i.e. HRup or HRdown) occur in motoneuron firing threshold and axonal conduction velocity, in several different synaptic terminal populations on the motoneuron, and probably in spinal interneurons as well [2,3,10,16].

The phase I and Phase II changes described in the present rat study, combined with recent work in the rat showing that the corticospinal tract is essential for H-reflex conditioning while other major descending tracts are not essential [4,7], imply that H-reflex conditioning is initiated by rapid mode-appropriate alteration in corticospinal tract influence and continues to develop due to the persistence of this alteration. Furthermore, the close similarity between rats and monkeys in phase I and phase II changes suggests that corticospinal tract influence is also responsible for H-reflex and SSR conditioning in the monkey. This is consistent with data indicating that SSR conditioning in humans is impaired by damage to sensorimotor cortex [11]. The present results contribute to the growing evidence that the phenomenon of SSR or H-reflex conditioning is similar in primates and rats. Therefore, they encourage work toward a single theoretical model and indicate the clinical relevance of the rat data.

We thank Ms Trish Herchenroder, Ms Dayna M. Stark, and Mr Hesham Sheikh for excellent technical assistance. This work was supported by grants from the National Institutes of Health (NS22189 (J.R.W.) and HD36020 (X.Y.C.)), the American Paralysis Association (X.Y.C.), the Paralyzed Veterans of America Spinal Cord Research Foundation (X.Y.C.), and the International Spinal Research Trust (J.R.W.).

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