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An Intelligent Flow Control System for Long Term Fluid Restriction in Small Animals

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Abstract— **Fluid retention is one of the most common symptoms in patients with chronic heart failure. Although fluid restriction may be a therapeutic strategy, the degree of fluid restriction necessary for the best therapeutic outcome remains unknown partly due to the lack of proper experimental method to restrict water consumption in small animals. The traditional methods that allow animals to access water only in a limited time window or within pre-determined daily volume can be stressful because the animals may become thirsty during the time of water deprivation. To provide a less stressful water restriction paradigm, we designed a feedback-control system of drinking flow to modulate the drinking behavior of small animals. This system consisted of an infrared droplet sensor for monitoring the drinking flow and a computer controlled electric valve to regulate the water availability. A light signal which synchronized with the command for opening the valve was set to establish a conditioned reflex. An animal test indicated that rats were adaptable to a precisely programmed water supply. This system may warrant investigation into the consequences of fluid restriction in chronic experimental animal study.**

I. Introduction

Fluid retention is one of the most common symptoms in patients with chronic heart failure (CHF). International guidelines recommend fluid restriction of 1 to 1.5 liter/day for CHF patients.[1-3] Although the fluid restriction may be a therapeutic strategy against fluid retention, evidence for the degree of fluid restriction necessary for the best therapeutic outcome is still lacking. Apparently, too severe fluid restriction can cause dehydration and oliguria, resulting in a deterioration of circulatory conditions. Therefore, it is difficult to quantify the relationship between the degree of fluid restriction and its therapeutic outcome in clinical settings. Translational studies may be a solution to elucidate the therapeutic effect of fluid restriction. Although many kinds of animal models have been established to investigate pathophysiology of human diseases, until recently, there has been little information available in the literature as to the long-term fluid restriction in experimental animals. The methods of fluid restriction applied in previous reports were manually limiting a time window of water supply [4] or daily volume consumption.[5] These methods can be stressful because the animals may become thirsty during the time of water deprivation.[6] Although a recent report used gel food for controlling water consumption,[7] this method necessitates a preparation of the gel mixture and the amount of fluid consumption is imprecise. To provide better water restriction paradigm that may be less stressful for animals, this study aimed to develop an automatic, programmable feedback-control system of water supply for small experimental animals.

II. Methods

A. The intelligent flow control system

Several droplets of water were consumed within a few minutes by a single episode of drinking. We refer this episode to as a drinking episode. We refer the intervals between the drinking episodes to as a drinking interval. We designed a 16-channel automatic feedback-control system of water supply that was comprised of a droplet sensor, a miniature electric valve and a personal computer with analog input and output interfaces (Fig. 1). The droplet sensor is an infrared motion detector which is used in an automatic infusion pump (FP-970, Matsuyoshi & Co.,Ltd.). A custom-made software counts the droplets in a tubing of water supply and records their time for off line analysis. It also has a capability of individualizing the drinking volume per episode and intervals between the drinking episodes by controlling the electric valve (LHLA0531111H, The Lee Company). The signal light (LED) is set near the nozzle and synchronized with the valve opening to indicate water availability.

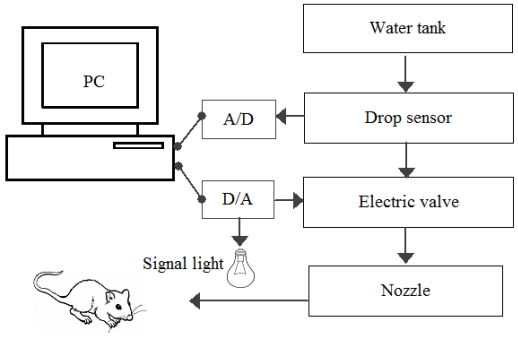

Figure 1. Schematic description of the flow control system. The signal light is synchronized with the electric valve.

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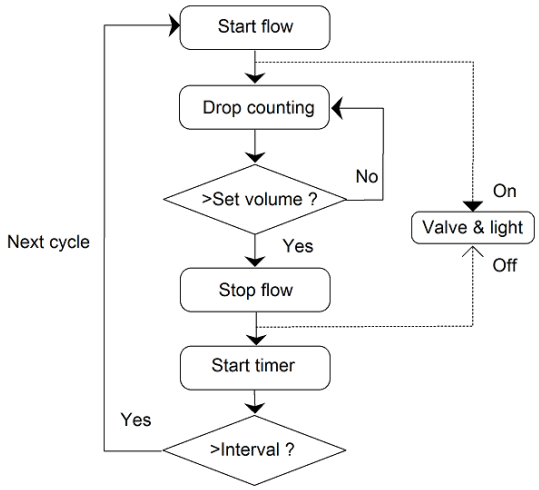

Figure 2. A simple flowchart representing the algorithm for limiting the drinking volume per episode.

B. Animals

The care and use of the animals were in strict accordance with the guiding principles of the Physiological Society of Japan. All protocols were reviewed and approved by the Animal Subject Committee in the National Cerebral and Cardiovascular Center, Japan. Male rats of Wistar strain were used (12 week old, body weight 250−300 g). The animals were housed individually in a standard cage. The rats remained in a temperature-controlled room (24 ± 2℃) illuminated between 6 A.M. and 6 P.M. The animals had free access to food (CLEA Rodent Diet CE-2) which contained 0.31% sodium and 1.03% potassium. Body weight of the animals was measured weekly.

C. Fluid restriction program

When starting the fluid restriction program, parameters such as the body weight, drinking interval, expected number of drinking times and targeted daily volume were assigned to individualize each drinking volume (EDV) according to the following equation

$$EDV = \frac{TDV \times BW}{ET}$$

where TDV is the targeted daily volume, BW is the body weight, ET is the expected drinking times. After the rats adapted the new cage environment with a new nozzle, the feedback control program was started. As shown in Fig. 2, the water becomes available after a predefined drinking interval. The rats can drink water until the number of droplets reached that equivalent to EDV. Although the rats are deprived of water if the amount of drinking reached EDV, the duration of water deprivation is at most the predefined drinking interval. This algorithm can shorten the duration of thirst compared to the traditional method of limiting a time window or daily water consumption.

D. Response time and nozzle access time

Because the present system precisely records the command for opening the valve and the time of each droplet, the time lag between the command and the first droplet caused by the animal drinking behavior can be used as a response time (RT, in s). The top panel of Fig. 3A is a typical example of drinking episode. The drinking episode clustered at the beginning of command signal. The bottom panel of Fig. 3A shows an expanded time scale for a single drinking episode. The horizontal arrow indicates RT. The RT may be an indicator of the establishment of the conditioned reflex between the light signal and the drinking behavior. To test whether the signal light helped establish the conditioned reflex, we turned off the light and examined the resulting RT distribution. In order to further examine the effect of the establishment of the conditioned reflex on animal activity, we connected a commercially available touch sensor (capacitive sensing circuit) to the nozzle and detected the time when the rat touched the nozzle. The touch sensor signals were collected at 10Hz sampling rate simultaneously with dropping signals.

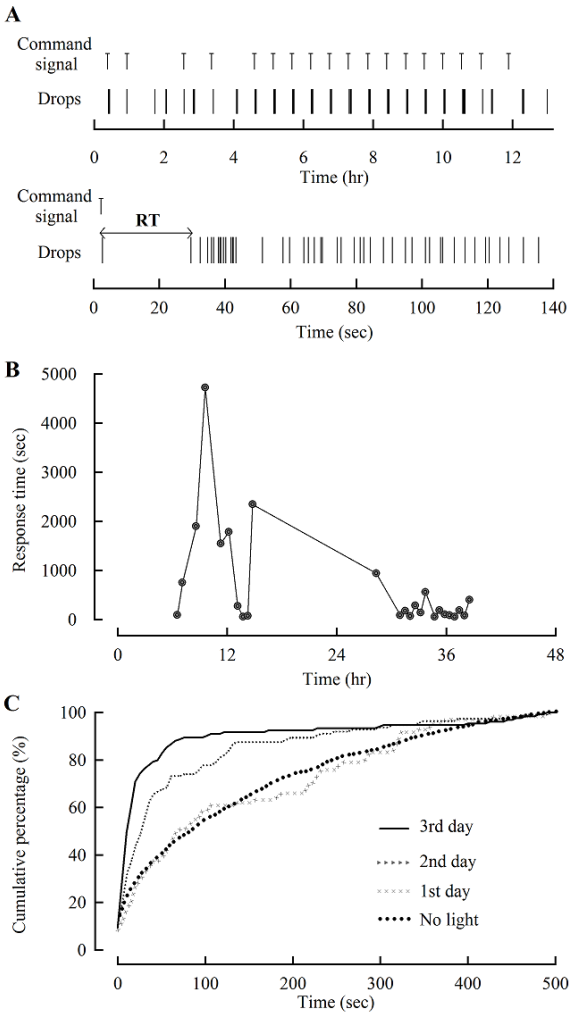

Figure 3. Adaptation of the rats to conditioned signal which indicates the electric valve states. (A) A sample of recorded data. Top is one day (13hr) drank 18 times; bottom is one dinking episode and shows response time (RT). (B) A typical recording of the RT of a rat at the first two days after feedback control of the drinking flow. (C) Distribution of the RTs of the rats without (n=4) or with the conditioned signal during the first 3 days (n=6) after fluid restriction.

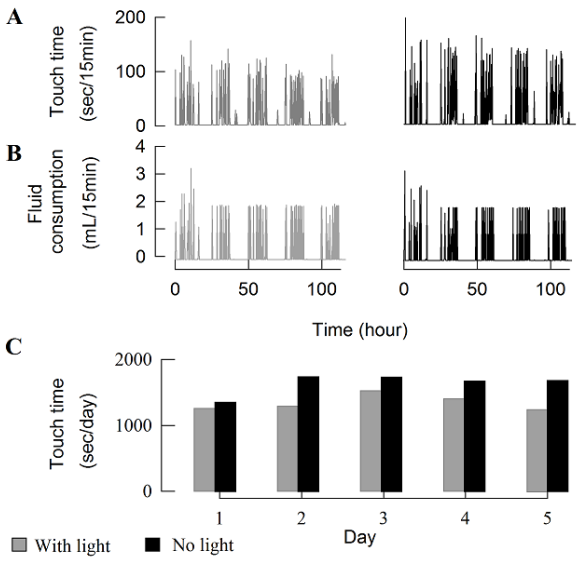

Figure 4. Drinking behavioral response of rats with (gray lines and bars) or without (black lines and bars) conditioning light signal. The first day was drinking ad libitum. (A) Nozzle access time by the rats recorded using a touch sensor. (B) Each drinking volume. (C) Daily cumulated touch nozzle time.

III. Results

A. Establishment of conditioned reflex

Most of the rats had adapted to the light signal synchronized with the valve opening within the first two days. Once the program started, RT became longer (Fig. 3B) at first several trials, then after 5-8 trials, 80% of the total RTs became less than 100 s in the second day, and 90% of the total RTs became less than 100 s in the third day (Fig 3C). Thereafter, the profile of RT was not changed markedly.

B. The effect of conditioned reflex on nozzle touching activity

Shown in Fig. 4 are nozzle access activity (Fig. 4A) and actual fluid consumption (Fig. 4B). When the restriction program started, animals increased the nozzle access time in both with and without conditioned light environments (Fig. 4C). At the fifth day with the conditioned signal, the rat reduced the access time to the level of free drinking. In contrast, the rat without the conditioned signal, nozzle access time kept longer than that of free drinking.

C. The effect of drinking behavior modulation on daily fluid consumption

This study limited the drinking volume per episode to 30–40% of the free drinking volume per episode and placed at least 30 min interval between the drinking episodes. This modulation decreased daily fluid consumption from 30.1 ± 2.0 to 21.0 ± 0.8 mL/day ($P < 0.01$) and increased the number of drinking episodes from 13.2 ± 1.3 to 20.8 ± 0.8 times ($P < 0.01$) (Fig. 5). Although the daily fluid consumption was decreased, there was no great influence on the circadian rhythm of the animal behavior. We had not limited the daytime (illumination on) access to the water, because the drinking interval was set even during daytime and nighttime Most of the rats, however, had kept their circadian rhythm that did not drink during the daytime. There was also no significant decrease of the body weight.

IV. Discussion

The present results showed that the automated control system worked effectively with respect to 1) limiting the researchers' need to visit the animal room, 2) ensuring the accurate recording of fluid consumption, and 3) reducing interventional stress. The animals established the conditioned reflex between the light signal and drinking behavior within a few days. The rats kept day-night rhythm and no abnormal behavior was observed during the programmed water restriction. The fluid consumption can be easily individualized by setting the program parameters.

The present study demonstrated the utility of a high resolution recording of water consumption and flow control system. Methodologically, this is the first proposal for the long-term fluid restriction by feedback controlling EDV in small animals. This technique has several advantages for gaining new insights into the pathophysiology associated with drinking behavior and water balance in various experimental disease models.

A. Thirst and thirst modulation for fluid restriction

Thirst is desire for drinking usually caused by dehydration to maintain water balance. The most reliable indicator of thirst is to measure the real drinking volume until the subject is satiated with drinking. In human clinical study, except for healthy subjects,[8] it is ethically unrealistic to force patients (such as CHF) drink too much or too less regardless of their drinking desire. Alternatively, thirst has been assessed by visual analog scale and categorical rating instruments. Both methods are trying to quantify the perception through individual explanation of the sensation subjectively. On the other hand, the real drinking volume measurement may be the only way to evaluate thirst in animal study. A few studies had measured cumulative fluid consumption by weighing the drink bottles as the indicator of thirst.[9] However, during ad libitum drinking, EDV is not constant for each drinking, and cumulative fluid consumption could not exactly reflect drinking desire at each moment. Even with the same daily ingestion volume in the same animal at different days or in different individuals, the number of drinking episodes may be different. If we assume that the animals drink water until they satiated, the EDV should be a more reliable index of moment-to-moment thirst in animal study.

We selected the droplet sensor and continuously recorded each dropping time, which made it possible to analyze the drinking behavior in various time scales. For instance, calculating the EDV and measuring daily water consumption were possible (Fig. 3A). Based on the high resolution recording technique, we were able to precisely feedback control EDV and set appropriate intervals between the drinking episodes. This approach may avoid those unexpected stresses like polydipsia that might occur in the previous reports using time window or volume limitation methods of fluid restriction.[10]

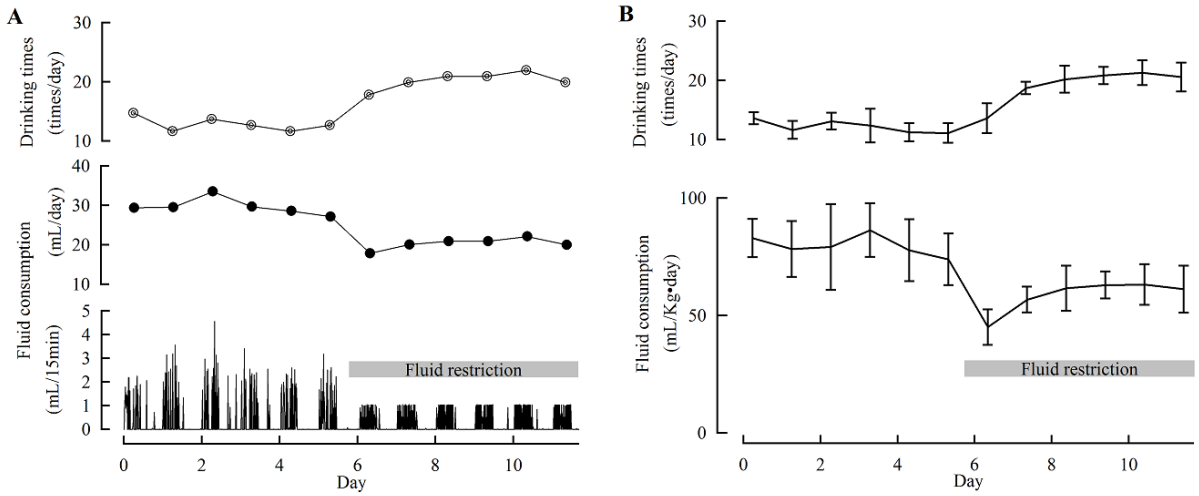

Figure 5. Effects of drinking behavior modulation on fluid consumption. (A) A representative recording of drinking behavior of a normal rat. (B) The responses of averaged fluid consumption to fluid restriction and drinking times in the rats. (n = 6)

B. Establishment of conditioned reflex to facilitate the animals for adaptation

The results of the present study showed that rats possess strong adaptability to establish conditioned reflex to the new environment stimulus in a short period. Previous studies have shown that rat is so smart and easily establishes various conditioned reflex.[8] It is reasonable that if there is no conditioned reflex, the thirsty rats have to try more times on the nozzle to access the water (Fig. 4). It will increase physical activity and fruitless work that results in fatigue and stress. Establishment of the conditioned reflex may alleviate stresses caused by the artificial intervention of water supply. These characteristics may be especially meaningful in the chronic experimental animal study.

This automatic system recorded the time of each droplet and the command for opening the valve. The RT may become a new indicator for an animal behavioral study. The series of RT (Fig 3A) after starting the program showed that rats adapted to the conditioned signal in the first day, and then completely established the reflex at the second day. Daily drinking volume reduced at the first day because the delayed RT decreased the number of actual drinking episode.

C. Advantages of the intelligent flow control system

The presented automatic system has realized simultaneous recording and controlling multi-channel drinking behavior. This system is precise enough for application in rat and mouse models, and can continuously record the drinking activity for months. The automation system will greatly improve efficiency of chronic animal study by reducing the daily needs of replacing water bottles. Future development that combined with urine collecting and recording systems is expected to enrich new physiological and pathophysiological knowledge on the area of water metabolism.

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