METHODS

Method of Evaluation of Dynamic Changes in Food Motivation in Rabbits during Successful Food-Procuring Behavior

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We developed a method for evaluation of dynamic changes in the level of food motivation in rabbits during successful food-procuring behavior. Quantitative and temporal parameters of food consumption over each successive cycle of food-procuring behavior were used. The amount of consumed food was converted into electrical signal using an electronic balance incorporated into a hardware-software complex. Temporal parameters of food-procuring activity were determined by changes in electric signal in real-time mode. This method makes it possible to record simultaneously food-procuring activity and impulse activity of masticator muscles and esophageal muscles, which enables parallel analysis of rhythms of food consumption, mastication, and swallowing.

Key Words: *level of food motivation; measuring method; food-procuring activity; quantitative and temporal parameters*

Measurement of the amount of food consumed during the experiment (a parameter depending on the duration of food deprivation) is a method of evaluation of food motivation in animals. The level of food motivation excitement decreases during successful food-procuring behavior and nutrient need satisfaction [8]. For evaluation of dynamic changes in the level of food motivation during nutrient need satisfaction, not only quantitative, but also temporal parameters of food consumption during each successive cycle of successful food-procuring behavior should be determined: from the start of eating to the end of consumption of easily available and preferable food.

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Objective methods of recording of temporal parameters of foraging behavior (tape recording and photoelectrical method) were primarily used for the studies of conditioned activity, *e.g.* during operant food-procuring task conditioning in rabbits [1,2]. Under these conditions, a strictly measured amount of food (3-5 g carrot) was used as reinforcement in each food-procuring cycle.

We found no published reports on studies where quantitative and temporal parameters of food consumption throughout the whole food-procuring act were simultaneously recorded by objective methods.

Here we developed a method for evaluation of dynamic changes in the level of food motivation in rabbits during successful food-procuring behavior.

The amount of consumed food was converted into electrical signal using a GF-600 electronic balance with a GX-06 analog (not digital!) output followed by their input into a channel of a MP100 hardware-software complex (Biopac Systems Inc.). Temporal parameters of food-procuring activity were determined by changes in electric signal in real-time mode. An advantage of this method is precise artifact-free recording of quantitative and temporal parameters of food-procuring activity of experimental animals under conditions of free behavior.

The plastic case of the balance was protected with a Plexiglas cover (33×24×9.5 cm) with 2 openings (Fig. 1): on the front wall (5×5 cm) above the control panel and on the top of the cover (diameter 9 cm) for a plastic feeding rack (weight 8.9 g, diameter 8.8 cm, and height 2.5 cm). The feeding rack was placed on the balance platform. This construction enables free movement of the rack up and down with the balance platform and prevents its side displacement when the rabbit takes the food. Pelleted food PK-92-390 (80 g) was placed into the rack as the food stimulus. The weight of each pellet is about 0.3 g.

The analog output of electron balance was connected via a UIM 100C commutation block to a measuring channel of MP100 hardware-software complex, which, in turn, was connected to a computer (Fig. 2). Analog electrical signal from the electron balance linearly depending on the food weight on the balance platform was recorded, processed, and visualized on the monitor using AcqKnowledge software. The accuracy of food weight measurement on the GF-600 electron balance in a MP100 complex was ±0.04 g. The main technical characteristics of the GF-600 balance with an analog output are the upper weight measuring limit of 600 g and the range of the output signal 0.1 V. The range of output voltage of GF-600 balance corresponds to the range of input voltage range of MP100 complex.

After standard AcqKnowledge software start-up procedure, the channel for recording of quantitative and temporal parameters of food consumption was calibrated. To this end, empty food rack was placed on the balance platform and the zero point (g) was set by pressing "re-zero" button on the control panel. In AcqKnowledge, a channel calibration window was opened, "Cal1" button was clicked, and a zero was set in "Scale value" box. After filling the rack with the food (80 g), "Cal2" button was clicked and "80" was put into the second row of "Scale value" box. Then, measurement units were chosen (g) and the channel calibration dialog box was closed. After that, an isoelectric line corresponding to food weight 80 g appeared on the monitor. Downward shift of this line by 1 mV corresponded to consumption of 0.6 g food. For programmed elimination of high-frequency electrical interference, low-frequency filter with a cutoff frequency of 0.3-0.5 was set.

During the experiment, the rabbits subjected to 1-, 2-, or 3-day food deprivation were placed into experimental camber with free access for pelleted food situated in the food rack on the platform of the electron balance (Fig. 1).

The successful food behavior is a chain of successive food-procuring cycles consisting of two phases:

Fig. 1. Recording of food-procuring behavior of a rabbit under conditions of free access to food in a rack placed on a platform of an electronic balance. *1*) GF-600 electronic balance; *2*) food rack with pelleted food; *3*) balance cover.

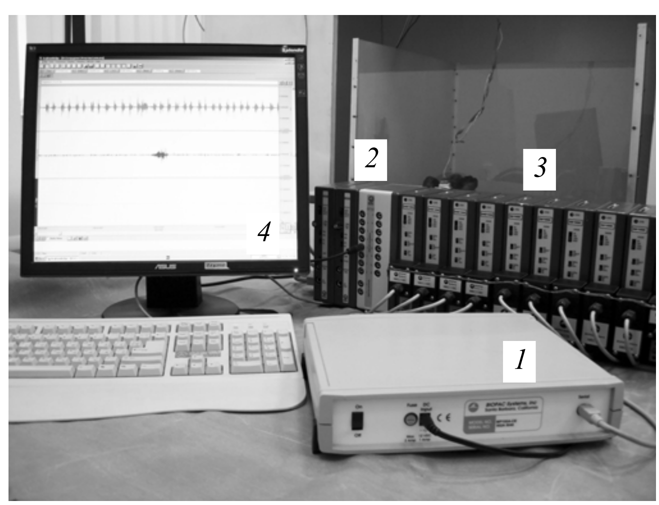

Fig. 2. MP100 software-hardware complex used for recording of food-procuring behavior in parallel with electrical activity of masticator muscles and esophageal muscles. *1*) MP100 microprocessor; *2*) UIM 100C commutation device; *3*) EMG 100C biopotential amplifier modules; *4*) display.

food take and mastication. The animal rapidly presses the balance platform during eating, which is seen on the monitor as a short positive electrical impulse (phase of food take). After that, the balance platform goes up, while the isoelectric line is shifted down to a value corresponding to the weight of consumed food and remains at this level until the next food take (phase of mastication). This pattern of foraging behavior remains until satiation (Fig. 3).

This method enables evaluation of temporal parameters of successive food-procuring cycles (periods and duration of food take and mastication phases) and quantitative parameters of food consumption in each cycle. Moreover, the rate of food-procuring activity (amount of consumed food over time unit) in the course of nutrient need satisfaction and attenuation of food motivation can be calculated.

The proposed method makes it possible to record simultaneously food-procuring behavior and impulse activity of masticator muscles involved into food take and mastication and muscles of the cervical portion of the esophagus participating in the swallowing act.

Continuous recording of electrical activity of masticatory muscles and muscles of the cervical portion of the esophagus in rabbits was performed during eating via chronically implanted electrodes [3-7]. Electrical signals from electrodes are inputted through a commutation device on EMG 100C biopotential amplifier and then to MP100 microprocessor and then digitized and displayed on the monitor using AcqKnowledge software. This enables simultaneous recording of electrical activity of striated muscles of the digestive tract and food-procuring activity of experimental animals.

Thus, the method proposed by us enables precise recording of the quantitative and temporal parameters of food consumption in rabbits in the dynamics of food-procuring behavior (from the start of eating to satiation), and, hence, to evaluate dynamic changes in food motivation during nutrient need satisfaction. An advantage of this method is its compatibility with recording of impulse activity of masticator muscles and esophageal muscles, which enables parallel analysis of food consumption, mastication, and swallowing rhythms.

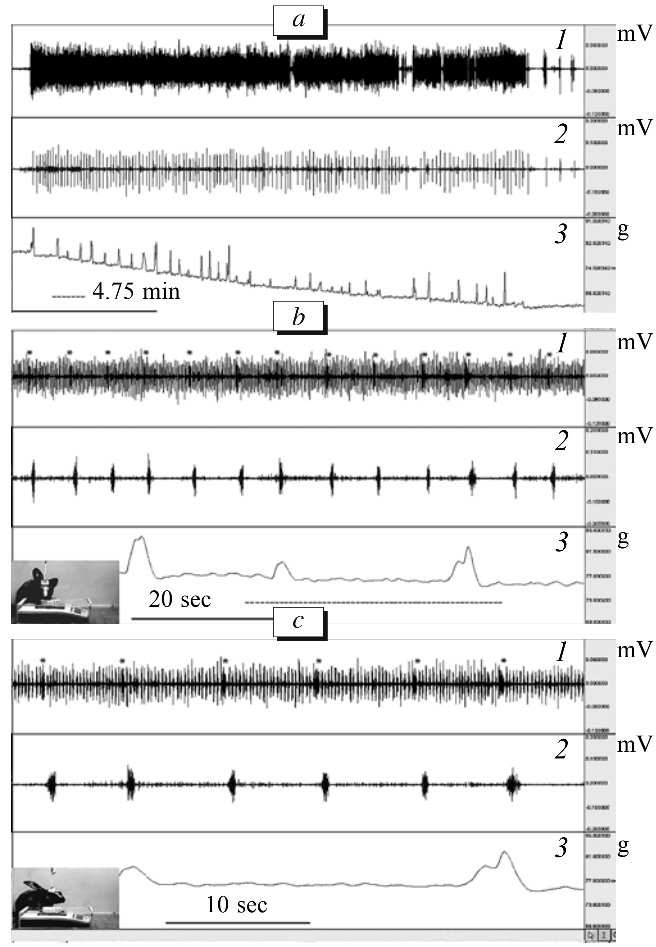

Fig. 3. Myoelectrical activity of the mylohyoid muscle (*1*) and cervical portion of the esophagus (*2*) and food behavioral reaction (*3*) of a rabbit subjected to 24-h food deprivation during successful food-procuring activity. *a*) scan velocity of a physiological process 0.3 mm/sec; dotted line underlines a record segment reproduced on fragment *b* at a rate of 4.3 mm/sec; *b*) dotted line underlines a record segment reproduced on fragment *c* at a rate of 8.6 mm/sec. *Spike bursts generated by mylohyoid muscle during swallowing. Positive electrical impulses on the analog curve of food behavioral reaction (*3*) correspond to food take, while the subsequent downward shift of the isoelectric line corresponds to the amount of food consumed during the food-procuring cycle. Lower left pictures: food behavior of the rabbit: during food take (*b*) and mastication (*c*).

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