Role of linkage between cerebral activity and baroreflex control of heart rate via central vasopressin V1a receptors in food-deprived mice

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ABSTRACT

We previously reported that cerebral activation at the onset of voluntary locomotion suppressed baroreflex control of heart rate (HR) and increased arterial pressure via vasopressin V1a receptors in the brain. Here, we examined whether these responses were associated with food seeking, a motivated behavior, using free-moving wild-type (WT, n=10), V1a receptor knockout (KO, n=9) and wild-type mice locally infused with a V1a receptor antagonist into the nucleus tractus solitarii (BLK, n=10). For 3 consecutive days mice were fed ad libitum (Fed), food deprived (FD), and refed (RF) under a dark/light cycle (19:00/7:00). Food was removed on day2 and restored on day3 at 18:00. Throughout the protocol, cerebral activity was determined from the power density ratio of θ- to δ-wave band (θ/δ) by electroencephalogram every 4sec. Baroreflex was evaluated by the cross-correlation function (R(t)) between changes in HR and arterial pressure every 4sec. The cerebro-baroreflex linkage was then evaluated by the cross-correlation function between θ/δ and R(t). Behavior was recorded with CCD camera. We found that cerebro-baroreflex linkage, enhanced in WT at night after FD (P=0.006), returned to Fed level after RF (P=0.68). Similarly, food-seeking behavior increased after FD to a level twofold higher than during Fed (P=0.004) and returned to Fed level after RF (P=0.74). However, none of these changes occurred in KO or BLK (P>0.11). Thus, the suppression of baroreflex control of HR linked with cerebral activation via V1a receptors might play an important role at the onset of motivated behaviors, such as food seeking induced by FD.

Keywords: cerebral activity, baroreflex, motivated behaviors, exercise, vasopressin

NEW & NOTEWORTHY

Motivated behaviors, characterized by goal-directed and persistent movements, are indispensable for living. However, how cerebro-cardiovascular adjustment occurs during such behaviors remains unknown. By focusing on food-seeking behavior in a food-deprived condition using free-moving mice, we found that this condition enhanced the linkage between cerebral activation and suppression of baroreflex control of heart rate through central vasopressin V1a receptors, making it easier to start motivated behaviors by enhancing pressor response.

INTRODUCTION

Motivated behaviors evoked by such sensations as hunger, thirst, and temperature are characterized by goal-directed and persistent movements for living by maintaining the homeostasis in the body (1). Although neural network mechanisms in the hypothalamus and limbic system have been extensively studied for specific behaviors (1-6), there have been no studies to evaluate how cardiovascular adjustment occurs at starting the behaviors in response to activation of the higher brain regions after receiving the signals from the hypothalamic and limbic regions.

In the previous studies (7, 8), we continuously measured cerebral activity with an electroencephalogram (EEG) and brain blood flow with a laser-Doppler flowmeter, together with heart rate (HR) and arterial blood pressure, in free-moving mice throughout the daytime. As a result, we found that the sensitivity of baroreflex control of HR was frequently suppressed at the time when the cerebral cortex was spontaneously activated, which was followed by voluntary locomotion with increased HR and arterial blood pressure. Because cerebral activation occurs sparsely during the daytime, which is the inactive phase for mice, we could make pairwise analyses between each incidence of transient cerebral activation and suppression of baroreflex control of HR which lasted for only a few sec or min (7, 8). However, it remains unknown how baroreflex control of HR is associated with cerebral activity during motivated behaviors which require a much longer time frame and occur during the nighttime, which is the active phase for mice.

To solve the problem and quantify the linkage between cerebral activity and baroreflex control of HR during the active phase, we used cross-correlation analysis in the present study. Using this approach, we examined the hypothesis that the linkage between cerebral activation and the suppression of baroreflex control of HR would be enhanced during the active phase for

mice when the motivated behaviors occurred. Moreover, we examined the hypothesis that central vasopressin V1a receptors would significantly contribute to any enhancement of the linkage, as we previously suggested that the receptors in the brainstem area were indispensable mediators for transmitting the command from the higher brain regions to the cardiovascular center to evoke the pressor responses before starting voluntary exercise (8).

To induce a possible enhancement of the linkage between cerebral activation and the suppression of baroreflex control of HR, we adopted a food-deprived (FD) condition since it was reportedly one of the most popular and easiest interventions for accelerating motivated behaviors (1). We thought that if the linkage between cerebral activity and baroreflex control of HR was enhanced in an FD condition, the finding would also occur in motivated behaviors for living other than eating.

METHODS

Animals

The generation of V1a receptor-deficient [i.e., knockout (V1a KO)] mice has been described previously (9). Mouse littermates not deficient in V1a receptors were used as wild-type controls (WT) for V1a KO mice in the first experiment and also used for treatment with V1a receptor blockade (V1a BLK) and vehicle control (CNT) in the second experiment (see below for protocol details). The genetic background of V1a KO and wild-type mice was a mixture of 129sv and C57BL/6J mice. Adult males of these mice were used for the study at 9-29 weeks of age. All mice were housed at 25˚C and 50% relative humidity with food or water ad libitum under a dark/light cycle (19:00/7:00). The procedures used were in accordance with the guiding principles for the care and use of animals in the field of physiological sciences published by the Physiological Society of Japan (2003) with prior approval of the Animal Ethics

Committee of Shinshu University School of Medicine. All animals were euthanized with a pentobarbital overdose at the end of the study.

Sample size

This is the first study to investigate the linkage between cerebral activity and baroreflex control of HR in an FD condition and compare the response between intact and V1a receptor-impaired mice. Therefore, we could not determine a sample size for the outcome based on 80% statistical power (1-β), α=0.05, an expected difference and an SD. Accordingly, in the present study, we adopted a sample size almost equal to that in our previous study (8), which compared the linkage between cerebral activity and baroreflex control of HR between intact and V1a receptor-impaired mice but in a fed ad libitum condition.

Preparations

WT and V1a KO mice in the 1st experiment

Before anesthetization of the mice, the body weights were 31.2±0.6 and 28.2±0.6 g for the WT (n=10) and V1a KO (n=9) mice, respectively. The V1a KO mice were significantly lighter than the WT mice (P=0.003). After anesthetizing the mice with pentobarbital sodium (50 mg/kg body weight, I.P.), we placed three stainless-steel screws (OD 1 mm) of EEG electrodes on the skull surface according to the following stereotaxic coordinates (11): AP -1.0 and L +1.0, AP -3.0 and L -1.0 mm from bregma, and AP +1.0 and L +1.0 mm from lambda in all mice (7). The screws in each mouse were fixed to the skull with dental cement. Then, a polyethylene catheter to measure mean arterial pressure (MAP) and HR was inserted into the left femoral artery so that the tip was positioned 5 mm below the left renal artery (10). The catheter was secured to the surrounding leg muscles. The arterial catheter and EEG electrodes were tunneled

subcutaneously and then exteriorized between the scapulae. The exteriorized catheter was connected to a cannula swivel (model TCS2-21; Tsumura, Tokyo, Japan), and the mouse was placed in a cage with a free-moving system (model TFM-170; Tsumura). The arterial catheter was flushed every day with 100 i.u. heparin in 0.2 ml saline. Surgery was performed at least a week before measurement (11).

CNT and V1a BLK mice in the 2nd experiment

Before anesthetization of the mice, the body weights were 32.1±0.5 and 31.1±0.8 g for the CNT (n=9) and V1a BLK (n=10) mice, respectively, with no significant difference between the groups (P=0.32). With the CNT and V1a BLK mice, we followed the same preparation procedure as in the first experiment, above. However, before fixing the EEG screws with dental cement, we inserted a stainless-steel cannula (OD 0.36, ID 0.18 mm) through the skull such that the tip was positioned in the nucleus tractus solitarii (NTS) (AP -3.2, L 0.0, and V +4.0 mm from lambda) (12) in the CNT and V1a BLK mice. The cannula was connected via 2 cm of silastic tubing to an Alzet osmotic pump (model 1002; Durect, Cupertino, CA, USA) that was placed in a subcutaneous cavity. The osmotic pump delivered either the 0.25-mM non-peptide V1a receptor-selective antagonist (OPC-21268; Tocris Bioscience, Ellisville, MO, USA) (8, 13) dissolved in 5% dimethyl sulfoxide (DMSO) / 95% artificial cerebrospinal fluid, or a vehicle solution consisting of 5% DMSO / 95% artificial cerebrospinal fluid at a rate of 0.25 μl/hr for 2 weeks into the NTS. The stainless-steel cannula in each mouse was also fixed to the skull with dental cement. The implantation was performed according to the method employed in previous studies (8, 14-16).

Protocol

WT and V1a KO mice in the 1st experiment

The experiment was conducted to investigate whether the linkage between cerebral activity and baroreflex control of HR was enhanced in an FD condition and whether V1a receptors were involved in the response. The WT and V1a KO mice were fed ad libitum (Fed), FD, and refed (RF), for 24 hr each, respectively, under a dark-light cycle for 3 consecutive days. Here, we used the terms 'dark' (19:00-7:00 or *hr* 0-12) and 'light' (7:00-19:00 or *hr* 12-24), respectively. Food was removed and restored at 18:00, 1 hr before the termination of the light phase on days 1 and 2, respectively. Throughout the protocol, we continuously measured cerebral activity with EEG together with HR, MAP and activity counts in free-moving WT and V1a KO mice. In addition, we recorded their behavior with a CCD camera.

CNT and V1a BLK mice in the 2nd experiment

The experiment was conducted to investigate whether V1a receptors in the NTS were responsible for linking cerebral activity and baroreflex control of HR to facilitate motivated behavior. The V1a receptor antagonist or vehicle was continuously infused into the NTS of wild-type mice using the osmotic pump. Except for the infusion, the protocol was the same as that of the 1st experiment. About a week after starting the infusion, EEG, HR, MAP and activity counts were continuously measured, and mouse behaviors were recorded in free-moving CNT and V1a BLK mice.

To confirm that the V1a receptor antagonist infused into the NTS did not leak into the peripheral circulation, we determined a change in MAP after an intra-arterial injection of arginine vasopressin (AVP) (V9879; Sigma-Aldrich, St Louis, MO, USA) dissolved in saline to evoke peripheral V1a receptor-mediated vasoconstriction. After the injection of 1 μg/kg AVP, MAP increased by 33.3±1.7 and 32.6±2.1 mmHg in the CNT and V1a BLK mice, respectively,

with no significant difference between the groups (P=0.81).

Measurements

EEG was measured through a bandpass filter of 0.5-30 Hz (Bioelectric Amplifier, model MEG-1200; Nihon Kohden, Tokyo, Japan). MAP was measured through a catheter connected to a pressure transducer (model TP-400T; Nihon Kohden). HR was counted from the analogue signal of the arterial pressure pulse with a tachometer (model AT-601G; Nihon Kohden) that calculated the inverse of the heart period taken from pulse wave maxima.

Activity was monitored with locomotion sensors located on a rectangular frame 25.5 x 18.5 cm in inner size (model LCM-10M; Melquest, Toyama, Japan) in which a mouse plastic cage 24.5 x 17.5 cm in outer size was placed. The sensors were composed of 3 pairs of infrared lamps and corresponding receivers on the longer frames and 2 more pairs on the shorter frames at ~6.3 cm apart from each lamp or receiver. The activity was expressed by the total count of times that mice crossed the beam every 30 sec.

Moreover, their behavior was always monitored with a CCD camera, and the relevant part was stored in an HDD (model PH-AQ-160/80GB; TEAC, Tokyo) within a visual data recorder (model AQ-VU; TEAC). The signal to store the movie was triggered by locomotion sensors (model LCM-10M). In this system, when the receiver's output was reduced below -0.09 V, the movie during the period from -15 sec to +60 sec of the trigger point was automatically stored in the HDD. Additionally, for the dark phase recording, mice were floodlit by an invisible infrared projector (wavelength 850 nm, model SM-50-940; Wireless Tsukamoto, Suzuka, Japan). Mice were connected to the measuring instruments at least 28 hr before the measurements.

Data acquisition

EEG, HR, MAP, and activity were digitized and stored in a computer (model Dimension 1100; Dell, Kawasaki, Japan) at 128 Hz with data acquisition software (Vital Recorder; Kissei Comtec, Matsumoto, Japan). HR and MAP were re-sampled at 10 Hz through a low-pass filter with an edge frequency of 1.5 Hz to remove pulsatile arterial pressure signals in order to determine baroreflex control of HR (see below).

Analyses

Outlines of analyses

To analyze a possible association between suppression of baroreflex control of HR linked with cerebral activation and a food-seeking behavior in an FD condition, we focused on the responses during the "dark phase" (**Table 1**). The reason was that during this phase, mice were more active and most of motivated behaviors reportedly occurred (1), which would make it easier to detect the association than it was in previous studies conducted during the light phase (7, 8).

Fig. 1 shows a typical example of θ/δ, R(t), ΔHR/ΔMAP, HR, MAP, and activity counts in a WT mouse for 12 hr of the dark phase in the FD condition (see below for details of θ/δ and R(t)). We found a few slow and periodic waves in θ/δ and R(t), each of which was accompanied by increases in HR, MAP, and activity counts. Therefore, as shown in **Fig. 2**, we performed auto-correlation analysis on θ/δ and R(t) in all WT and CNT mice (see below and **Table 2** for further details) to determine the periodic time of the waves. As a result, we found in the FD condition that the increases in θ/δ and R(t) occurred at the similar periodic times of 3.1±0.8 (SD) hr (range, 2.0–4.3 hr) and 3.1±0.7 hr (2.0–4.3 hr), respectively, but the average R(t) value decreased gradually with decreases in HR, MAP, and voluntary activity (**Fig. 1**), which was assumed to be one of the behavioral defense mechanisms for minimizing energy loss during

starvation (17). Accordingly, we performed the cross-correlation analysis between θ/δ and R(t) for the first 6 hr of the dark phase in the Fed, FD, and RF conditions to evaluate the linkage between cerebral activity and the baroreflex control of HR while focusing on the 1st and 2nd waves in all WT and CNT mice (**Table 3**). Moreover, we performed the behavioral analysis for the first 2 hr of the dark phase while focusing on the 1st waves, where the most prominent increases in R(t), HR, and MAP occurred in the FD condition in almost all WT and CNT mice (**Fig. 3**).

Finally, to examine whether V1a receptors were involved in these responses, we performed the auto/cross-correlation and behavioral analyses in all V1a KO and V1a BLK mice in the same way as those in the WT and CNT mice.

To confirm whether any enhancement of cross-correlation analysis between θ/δ and R(t) in the FD condition was due to real physiological responses and not random interactions, we adopted a surrogate data approach (see **Supplemental Methods and Results** for further details (https://figshare.com/s/a18a29dc9d34971cf50d)).

Cerebral activity analysis

We used the power density ratio of θ to δ wave band (θ/δ) in the EEG as an index of cerebral activity. To determine θ/δ, we calculated the power density every 4 sec in two frequency bands: δ (0.75-4.0 Hz) and θ (6.0-9.0 Hz).

Baroreflex control of HR analysis

More details of the analyses were previously reported (10, 11, 18). Briefly, the slope of ΔHR/ΔMAP was determined from the HR response to the spontaneous change in MAP every 4 sec using the cross-correlation function (R(t)). As shown in **Fig. 1**, R(t) above (red) and below

(blue) the lines of P=0.05 indicate significantly positive and negative correlations, respectively, which were used to determine positive (red) and negative (blue) ΔHR/ΔMAP. The formulae used for analyses are as follows:

$$R(t) = f(\Delta x(t+\Delta t), \Delta y(t)),$$

$$\Delta x(t) = x(t) - \bar{x}(t), \quad \Delta y(t) = y(t) - \bar{y}(t),$$

$$\bar{x}(t) = \frac{1}{\tau}\int_{t-\frac{\tau}{2}}^{t+\frac{\tau}{2}} x(t)\,dt, \quad \bar{y}(t) = \frac{1}{\tau}\int_{t-\frac{\tau}{2}}^{t+\frac{\tau}{2}} y(t)\,dt,$$

where R (t) is the cross-correlation coefficient between x (= MAP) and y (= HR) at the given time (t) after correction for the delay time (Δt = 0.6 sec) of HR response to MAP change. The $\bar{x}$ (t) and $\bar{y}$ (t) were averaged values of MAP and HR, respectively, from time t-$\frac{\tau}{2}$ to t+$\frac{\tau}{2}$ (τ = 4 sec). The slope of ΔHR/ΔMAP was determined every 4 sec after R(t) was confirmed as significant.

To assess the linkage between cerebral activity and baroreflex control of HR, we used R(t) as an index of baroreflex control of HR in the present study, while the slope of ΔHR/ΔMAP has been generally used. We chose to use R(t) for the following reasons: 1) ΔHR/ΔMAP was determined only when R(t) was significant, resulting in ΔHR/ΔMAP that was not a continuous variable, while R(t) was determined every 4 sec so that it could be used for the cross-correlation analyses between θ/δ and baroreflex control of HR as a continuous function of time (see below) to evaluate their linkage; 2) we confirmed that ΔHR/ΔMAP was positively correlated with R(t) that was transformed to $Z_{R(t)}$ (see below) during all 3 days for all groups (all, P<0.001) in the present study and also in previous studies (7, 8).

Auto- and cross-correlation analyses

We performed the auto-correlation analysis using θ/δ and R(t) in the Fed and FD conditions

for the 12 hr dark phase. As shown in **Fig. 2**, we found that the peak values and the amplitude between the peak and valley values were higher in the FD condition than in the Fed condition and, moreover, that the time to peak of θ/δ was almost identical to that of R(t) in the FD condition while it deviated somewhat in the Fed condition in almost all WT and CNT mice. Therefore, to assess any change in the linkage between θ/δ and R(t) by FD in all groups of mice, we determined the number of mice meeting three criteria for both θ/δ and R(t): 1) significant peaks of auto-correlation function at $P<0.001$ appeared in a range of time shift from 0 to 6 hr; 2) its amplitude (a difference between peak and valley values) was >0.085 which was a minimal value of 2 SD of auto-correlation function in the FD condition in all WT and CNT mice; and 3) a significant peak of R(t) meeting the first criterion appeared within ±7 min from the first peak of θ/δ, of which the range was the closest to but out of 1 SD of the difference in the peak time between θ/δ and R(t) in the FD condition in all WT and CNT mice. The reason we focused on the first peak was that it was the highest in most of the WT and CNT mice. We performed the auto-correlation analysis after transforming R(t) to $Z_{R(t)}$ according to the following equation (19):

$$Z_{R(t)} = \frac{1}{2} \log_e \left[\frac{1 + R(t)}{1 - R(t)} \right]$$

This transformation was also done when we performed the cross-correlation analysis between θ/δ and R(t). As shown in **Supplemental Fig. S1** (https://figshare.com/s/a18a29dc9d34971cf50d), the cross-correlation analysis was performed from *t*-2 hr to *t*+2 hr (3601 values) while moving *t* by an increment of 4 sec (but to simplify the figure, an increment of 1 hr has been schematically presented). Additionally, the auto-correlation and cross-correlation coefficients from these analyses were determined after the transformation to analyze them quantitatively, and the results are summarized in **Tables 2 & 3**, respectively.

Behavioral analysis

We used a visual data recorder viewer program (model AQ View1.0.2; TEAC) installed on a computer (model Latitude D530; Dell) to replay the movie that was stored in the HDD. Mouse behaviors were classified into the following categories: walking, sniffing, eating, drinking, grooming and others. The behaviors were then partially combined and reclassified into the following categories: walking and sniffing as "food-seeking behavior", eating as "eating behavior" and drinking, grooming and others as "other behaviors". The duration of each behavior was calculated for each mouse. For this analysis, walking or sniffing for more than 10 sec was defined as "food-seeking behavior" but that for less than 10 sec was "other behaviors," in order to exclude any coincidental actions and thereby extract their intentional behaviors. These results are shown in **Fig. 3**.

Statistics

Values are expressed as the mean ± SE. A Fisher's exact probability test was used to examine any significant differences in the number of mice meeting the criteria for synchronized periodic waves between the Fed and FD conditions or between the groups (**Table 2**). One-way ANOVA was used to examine any significant differences in the change in the cross-correlation between θ/δ and R(t) after FD (**Figs. 4 A&B**) and the change in food-seeking behavior after FD (**Figs. 4 C&D**) between the groups. Two-way [group x time] ANOVA for repeated measures was used to examine any significant differences in θ/δ, R(t), HR, MAP and activity counts from the Fed condition (**Table 1**), cross-correlation between θ/δ and R(t) from the Fed condition (**Table 3**), and mice behavioral analysis from the Fed condition (**Fig. 3**) between the groups. Subsequent post hoc tests to determine significant differences in the various pairwise comparisons were performed using the Tukey-Kramer test. All P values <0.05 were considered

significant.

RESULTS

θ/δ, R(t), HR, MAP, and activity counts

Table 1 shows θ/δ, R(t), HR, MAP, and activity counts for 3 days in the Fed, FD, and RF conditions, respectively, for the first 6 hr of the dark phase (i.e., 19:00 to 1:00) in the WT and V1a KO groups (*upper*) and in the CNT and V1a BLK groups (*lower*). θ/δ and MAP were similar between the Fed and FD conditions in all groups (all, $P>0.21$) except for MAP in the V1a BLK group, while R(t) and HR were significantly lower in the FD condition than in the Fed condition in all groups (all, $P<0.001$) but with no significant interactive effects of [group x time] on R(t) or HR (all, $P>0.11$). Activity counts tended to be higher in the FD condition than in the Fed condition for all groups but with no significant interactive effect of [group x time] (both, $P>0.52$). The significantly lower R(t) and HR tended to return to the baselines in the Fed condition after RF in all groups except for HR in the V1a KO group.

Typical examples of the measurements

Fig. 1 shows a typical example of θ/δ, R(t), ΔHR/ΔMAP, HR, MAP, and activity counts in a WT mouse for all 12 hr of the dark phase in the FD condition. As shown in the figure, R(t) increased (was less negative) as θ/δ increased, which was accompanied by increases in HR, MAP and activity counts. Because these variables appeared to show some periodic waves, we performed the auto-correlation analysis on θ/δ and R(t).

Auto-correlation analysis on θ/δ and R(t)

Fig. 2 shows a typical example of the auto-correlation analysis on θ/δ and R(t) with significant correlations at P<0.001 in a range of time shift from 0 to 6 hr in the WT mouse of **Fig. 1** in the FD, together with that in the Fed condition. Although the time to peak of θ/δ deviated slightly from that of R(t) in the Fed condition (*left* panel), they were identical in the FD condition (*right* panel) with higher amplitudes of fluctuations than in the Fed condition. Accordingly, in the auto-correlation analysis on both θ/δ and R(t), we determined the number of mice meeting the three criteria stated above.

As shown in **Table 2**, although the number of mice meeting all three criteria was few in the Fed condition in all groups, it increased significantly in the FD condition in the WT and CNT groups (both, P<0.001), while the increases were abolished in the V1a KO and V1a BLK groups (P=0.24 and P=0.50, respectively) with significant differences between the WT and V1a KO groups (P<0.001) and also between the CNT and V1a BLK groups (P<0.001). This was due to the increased number of mice meeting criterion 3) in the FD condition in the WT and CNT groups (vs. Fed condition, P=0.003 and P=0.001, respectively). These results suggest that although θ/δ and R(t) showed the periodic waves in the Fed condition, the two waves were more synchronized in the FD condition in the WT and CNT groups, but this synchronization in response to FD was abolished in the V1a KO and V1a BLK groups. Accordingly, we performed the cross-correlation analysis between θ/δ and R(t) to further evaluate the linkage between cerebral activity and the baroreflex control of HR in each group.

Cross-correlation analysis between θ/δ and R(t)

As shown in **Table 3**, we found that the FD condition increased the cross-correlation between θ/δ and R(t) by 53% in the WT group and 49% in the CNT group compared with the baselines in the Fed condition (P=0.006 and P=0.031, respectively), but it returned to the

baselines after RF (P=0.68 and P=0.14, respectively). By contrast, we found no significant increases in the cross-correlation in the V1a KO or V1a BLK group (P=0.58 and P=0.85, respectively). There were significant interactive effects of [group x time] on the cross-correlation in the FD condition between the WT and V1a KO groups (P=0.021) and between the CNT and V1a BLK groups (P=0.045).

Furthermore, using the surrogate data analysis, we confirmed that the increased cross-correlation between θ/δ and R(t) in the FD condition was due to real physiological responses of R(t) linking more with θ/δ, and not random interactions between them (see **Supplemental Methods and Results, Supplemental Table S1** for further details (https://figshare.com/s/a18a29dc9d34971cf50d)).

Food-seeking behavior

Fig. 3 shows durations of food-seeking, eating and other behaviors for the first 2 hr of the dark phase (i.e., 19:00 to 21:00) in the Fed, FD and RF conditions, respectively. In the WT group (**Fig. 3A** *upper*), food-seeking behavior increased in the FD condition, which was twofold higher than that in the Fed condition (P=0.004), and returned to the baseline after RF (P=0.74), whereas in the V1a KO group no significant increase in the behavior was observed in the FD condition (P=0.29) (**Fig. 3A** *lower*). There was a significant interactive effect of [group x time] on the food-seeking behavior in the FD condition between the groups (P=0.008). On the other hand, there were no significant interactive effects of [group x time] on eating or other behaviors in the FD condition between the WT and V1a KO groups (P=0.62 for eating, P=0.72 for other behaviors).

Similarly, in the CNT group (**Fig. 3B** *upper*), food-seeking behavior increased in the FD condition, which was threefold higher than that in the Fed condition (P=0.001), and returned to

399 the baseline after RF (P=0.99), whereas in the V1a BLK group, no significant increase in the
400 behavior was observed in the FD condition (P=0.11) (**Fig. 3B** *lower*). There was a significant
401 interactive effect of [group x time] on the food-seeking behavior in the FD condition between the
402 groups (P=0.048). On the other hand, there were no significant interactive effects of [group x
403 time] on eating or other behaviors in the FD condition between the CNT and V1a BLK groups
404 (P=0.55 for eating, P=0.30 for other behaviors). Thus, the increase in the food-seeking
405 behavior in the FD condition was particularly abolished in both V1a KO and V1a BLK groups.
406 **Fig. 4** shows the changes in the cross-correlation between θ/δ and R(t) and in the
407 food-seeking behavior during the dark phase by FD. The increase in the cross-correlation
408 between θ/δ and R(t) was significantly greater in the WT group than in the V1a KO group
409 (P=0.021) (**Fig. 4A**), which was accompanied by a significantly greater increase in food-seeking
410 behavior in the WT group than in the V1a KO group (P=0.038) (**Fig. 4C**). Similarly, the
411 increase in the cross-correlation between θ/δ and R(t) was significantly greater in the CNT group
412 than in the V1a BLK group (P=0.045) (**Fig. 4B**), which was accompanied by a significantly
413 greater increase in food-seeking behavior in the CNT group than in the V1a BLK group
414 (P=0.039) (**Fig. 4D**). These results indicate that the linkage between cerebral activation and the
415 suppression of baroreflex control of HR was enhanced by FD with enhanced food-seeking
416 behavior in the WT and CNT groups, whereas these enhancements were abolished in the V1a
417 KO and V1a BLK groups.
418
419 **DISCUSSION**
420 To our knowledge, this is the first study that has evaluated the baroreflex control of HR in
421 response to cerebral activation during motivated behaviors characterized by goal-directed and
422 persistent movements for living. The major findings in the present study are that the linkage

423 between cerebral activation and the suppression of baroreflex control of HR was enhanced by FD
424 with increased food-seeking behavior in intact mice while the enhancements were abolished in
425 V1a KO and V1a BLK mice.
426
427 **Differences between voluntary movements and motivated behaviors**
428 Previous studies reported that baroreflex control of HR was suppressed in cats when they
429 were confronted with aggressive individuals (20) and in rats of different strains (21). Moreover,
430 in two of our own previous studies we found that in free-moving mice, a transient increase in
431 cerebral activity lasting for a few sec or min suppressed baroreflex control of HR (7, 8).
432 Recently, we reported similar responses in humans when they intended to start exercise, which
433 were followed by enhanced muscle blood flow and oxygen consumption rate at the onset of
434 exercise (22). Thus, these responses are thought to be one of the feed-forward mechanisms for
435 adjusting cardiovascular functions for starting voluntary exercise smoothly (23).
436 On the other hand, the cardiovascular adjustment during motivated behaviors examined in
437 the present study may be distinguished from that during voluntary movements stated above.
438 The reasons are that the appetite for food is controlled by a “feeding center” and a “satiety center”
439 in the hypothalamus, which evokes eating and cessation of eating, respectively (2). When the
440 level of glucose utilization of the cell in the center is reduced such as in the FD condition, the
441 feeding center is activated and animals feel hungry enough to start eating (1). Inversely, when
442 the utilization is enhanced, the satiety center is activated. Additionally, the limbic system is
443 also involved in the regulation of appetite through a different neural pathway (3). Similarly,
444 osmo- or thermo-sensitive neurons in the hypothalamus are known to be involved in drinking
445 and thermoregulatory behaviors, respectively (4-6). Thus, the motivated behaviors may be
446 different from the voluntary movements in two important ways: they are evoked when the levels

of the homeostatic variables of the extracellular fluid have deviated from given ranges, and they occur in a burst fashion and continue over a long time frame until their levels have returned to the given ranges.

Therefore, in the present study, we sought to examine any linkage between the cerebral activation and baroreflex control of HR during the motivated behaviors which occur during the active/dark phase. However, we could not determine the relationship between a given transient increase in cerebral activity and the following suppression of baroreflex control of HR by pairwise comparisons as done in previous studies conducted during the inactive/light phase (7, 8), because the cerebral and baroreflex responses occurred much more frequently and sometimes not-intermittently, making it difficult to identify the incidence of each pair. To solve the problem and quantify the linkage between cerebral activity and baroreflex control of HR during the motivated behaviors, we performed cross-correlation analysis while using θ/δ and R(t) as an index of current status of cerebral activity and baroreflex control of HR, respectively, in the present study (see **Supplemental Discussion** for the significance and reliability of θ/δ and R(t) determination (https://figshare.com/s/a18a29dc9d34971cf50d)). As a result, we successfully determined the linkage between them after confirming their synchronization in response to FD by auto-correlation analysis (**Table 2**, **Fig. 2**).

Linkage between θ/δ and R(t), food-seeking behavior, and central V1a receptors

We found that in the intact mice groups, the cross-correlation function between θ/δ and R(t) was enhanced during the active/dark phase in the FD condition compared with that in the Fed condition (**Table 3, Fig. 4 A&B**), accompanied by an increase in the food-seeking behavior (**Fig. 3, Fig. 4 C&D**). However, these enhancements disappeared in the V1a KO and V1a BLK groups (**Table 3, Figs. 3&4**).

It has been suggested that the central V1a receptors in the NTS in the medulla were involved in controlling the feedback gain of baroreflex in the cardiovascular center by signals from higher brain regions (9, 24-26) while in these studies, baroreflex sensitivity was determined only once, either under anesthesia or after a short recovery from surgery. Recently, we performed continuous measurements of cerebral activity and the sensitivity of baroreflex control of HR during the inactive/light phase in free-moving V1a KO and V1a BLK mice which were prepared similarly to those used in the present study (see **Supplemental Discussion** for the validity of the blockade infusion protocol (https://figshare.com/s/a18a29dc9d34971cf50d)), and suggested that the linkage between cerebral activation, the suppression of the baroreflex, and the following voluntary movement was nearly abolished when V1a receptors were impaired (8). Those results suggested that the central V1a receptors were involved in the suppression of baroreflex control of HR at the onset of voluntary movement.

In the present study, we newly found that the mechanisms were activated during the active/dark phase in the FD condition, but this activation did not occur when the central V1a receptors were impaired (**Tables 2 & 3**, **Figs. 3 & 4**). Thus, V1a receptors in the NTS might be involved during motivated behaviors, such as food seeking, by facilitating the suppression of baroreflex control of HR in response to voluntary cerebral activation during the active/dark phase.

Although we found the reduced food-seeking behavior response to the FD condition in the V1a receptor-impaired mice compared with the intact mice groups, there were no significant reductions in eating behavior for the Fed condition in the groups (P=0.35-0.41) (**Fig. 3**). This might be because animals could easily get food with less intention during their free movement in the condition where food was scattered on the floor of a cage. These results suggest that motivated behavior which required more intention to move towards the goal was particularly

impaired in the V1a receptor-impaired mice groups.

In addition, it has been reported that stimulation of V1a receptors in the amygdala of the limbic system enhanced emotional reactions to external stress (27) and suppressed baroreflex sensitivity (20, 21), suggesting that no enhancement in cross-correlation between θ/δ and R(t) in the FD condition in the V1a KO group might be due to reduced emotional reactions to external stress by FD through V1a receptors in other brain regions. However, since we found no enhancement in cross-correlation between θ/δ and R(t) in the FD condition in the V1a BLK group or in the V1a KO group (**Table 3, Fig. 4 A&B**), the effects of V1a receptors in other brain regions on the linkage between θ/δ and R(t) in the FD condition would be minor, if any.

Finally, there have been a few studies suggesting that the activation of central V1a receptors suppresses eating behavior (28, 29), which seems to contradict the results of the present study. However, this discrepancy might be caused by different methods of food-related evaluation. The previous studies (28, 29) considered the amount of food consumed when plenty of food was provided, whereas in the present study we looked at the central pressor response to start food-seeking behavior by FD, which required more intention to move towards the goal and was not limited to food-related behavior.

These results suggest that the awareness of hunger by the cortical regions after receiving the metabolic signals from the hypothalamic and limbic regions in the FD condition suppressed baroreflex control of HR to evoke pressor responses through central V1a receptors, thus making it easier to start the food-seeking behavior.

The effects of FD on average values of R(t), HR, and MAP

As shown in **Table 1**, the average values of R(t) and HR were significantly reduced in the FD condition compared with those in the Fed condition in all groups of mice, while the

519 significant reductions disappeared in the RF condition, except for HR in the V1a KO group.
520 This might have been due to a reduced baseline metabolic rate in the FD condition which is
521 reportedly caused by the mechanisms to save energy expenditure during starvation in small
522 animals (17). However, the mechanisms are unlikely associated with the dynamic response of
523 R(t) linking with θ/δ in the FD condition through central V1a receptors in the present study since
524 there were no significant interactive effects of [group x time] on R(t) or HR between any groups
525 (**Table 1**), and also since the average MAP values in the FD condition were maintained at a level
526 similar to that in the Fed condition in all groups except the V1a BLK group, with no significant
527 interactive effects of [group x time] on MAP between any groups (**Table 1**). Thus, the reduced
528 baseline R(t) and HR by FD might not affect the dynamic response of baroreflex control of HR
529 to the transient increase in cerebral activity.
530
531 **Methodological considerations**
532 There are four main methodological considerations that deserve additional discussion.
533 First, although we used R(t) as an index of baroreflex control of HR in mice, we cannot provide
534 its translational value to humans in the units of beats/min/mmHg because prerequisites for
535 spontaneous baroreflex sensitivity calculations have not been well established in mice (30, 31)
536 compared with humans (32-35). Additionally, traditional spontaneous baroreflex methods are
537 reported to have limitations in detecting a change in sensitivity during the anesthesia-induced
538 unconscious state (36). However, the present study was conducted in conscious freely moving
539 mice, and moreover, we previously confirmed that R(t) was clearly dependent on intact carotid
540 baroreceptors using freely moving mice before and after peripheral baroreceptor denervation
541 (10).
542 Second, from the measurements in the present study, it is difficult to precisely distinguish

signals evoked by motivated behavior from those by voluntary movements. However, by adopting the FD condition to accelerate motivated behavior, we found that this condition enhanced the cross-correlation function between θ/δ and R(t) with increased food-seeking behavior, suggesting that central suppression of baroreflex control of HR was involved in motivated behavior.

Third, the changes in activity counts by FD were not significantly different between the intact and V1a receptor-impaired mice groups (**Table 1**). This might be because activity counts included both food-seeking and other behaviors and, thus, did not exactly reflect food-seeking behavior alone. However, by using a CCD camera, we clearly found that the duration of food-seeking behavior was increased by FD in the intact mice groups, while the increase was abolished in the V1a receptor-impaired mice groups (**Fig. 3, Fig 4 C&D**).

Fourth, the baseline values of cross-correlation between θ/δ and R(t) in the Fed condition for the 2nd experiment tended to be lower than those for the 1st experiment (**Table 3**), which might be due to non-specific effects caused by intracranial micro-infusion (see **Supplemental Discussion** for further details (https://figshare.com/s/a18a29dc9d34971cf50d)). However, we found similar responses of the variable to FD in the 1st and 2nd experiments, suggesting that these results do not bias the conclusions of the present study.

In conclusion, the linkage between cerebral activation and the suppression of baroreflex control of HR was enhanced with food-seeking behavior during the active/dark phase in the FD condition. Since the enhancements were abolished in V1a KO and V1a BLK mice lacking a mediator to evoke suppression of baroreflex after cerebral activation, the central pressor response via V1a receptors might play an important role in starting motivated behaviors, such as food seeking, during the active/dark phase in the FD condition.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.S., S.M., and H.N. were responsible for conception and design of research; E.S. and S.M. performed experiments; E.S. and S.M. analyzed data; E.S., S.M., and H.N. interpreted results of experiments; E.S. and S.M. prepared figures; E.S., S.M., and H.N. drafted the manuscript; E.S., S.M., and H.N. edited and revised the manuscript; E.S., S.M., and H.N. approved the final version of the manuscript.

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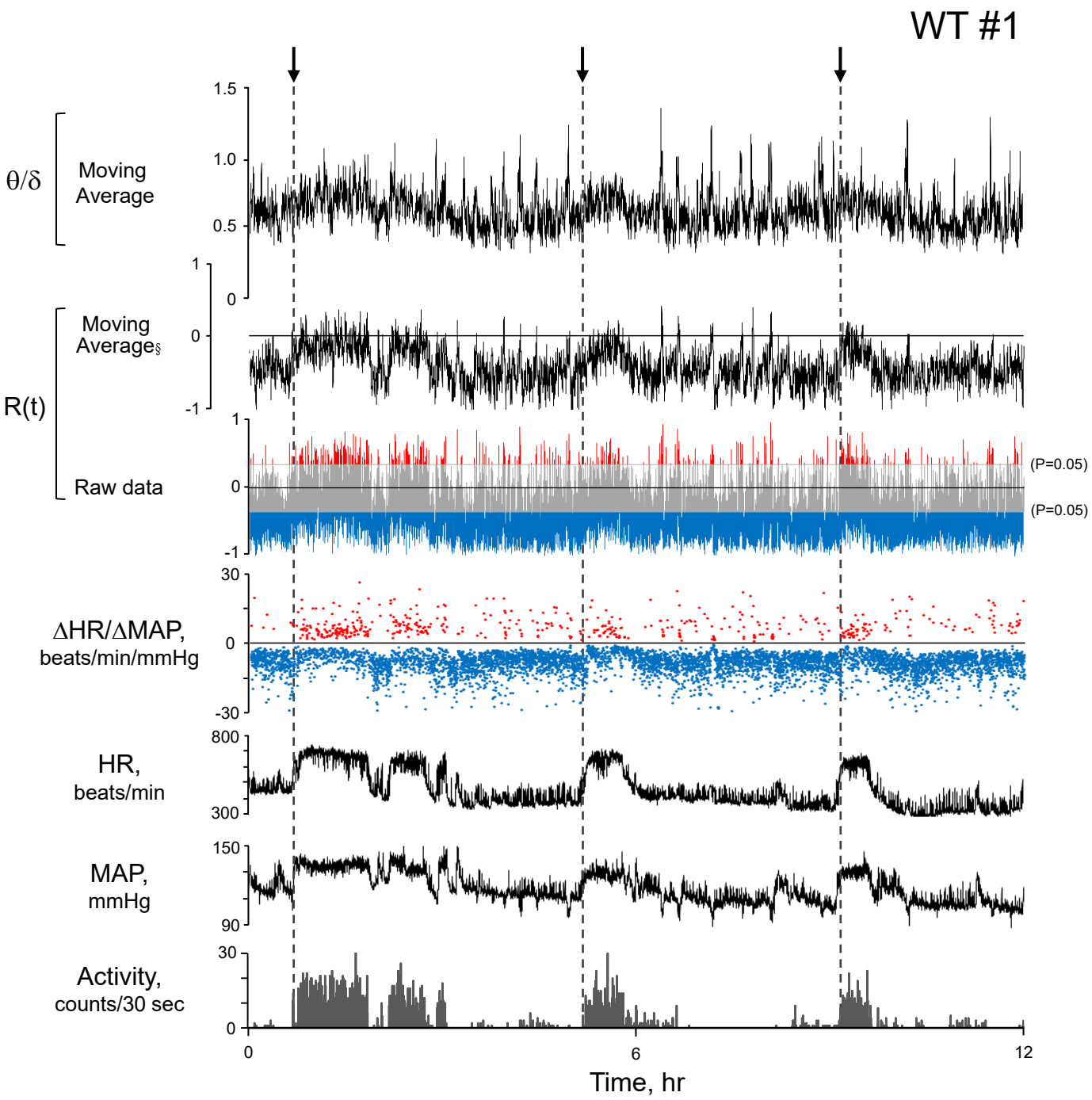
FIGURE LEGENDS

Fig. 1: A typical example of measurements of a wild-type control (WT) mouse (WT #1) for 12 hr of the dark phase in the food-deprived (FD) condition. Top to bottom: ratio of θ to δ wave band in the EEG (θ/δ), cross-correlation function (R(t)) between ΔHR and ΔMAP, ΔHR/ΔMAP, HR, MAP, and activity counts. R(t) above (red) and below (blue) lines of P=0.05 indicate significantly positive and negative correlations, respectively, during which period positive (red) and negative (blue) ΔHR/ΔMAP were determined, respectively. θ/δ and R(t) were determined every 4 sec and then averaged for a period from *t*-12 to *t*+12 sec (7 values) while moving *t* by an

731 increment of 4 sec. These values were used to perform the auto-correlation analysis on θ/δ and
732 R(t) (**Fig. 2, Table 2**) as well as the cross-correlation analysis between θ/δ and R(t) (**Table 3**).
733 § Average values after z transformation.
734
735 **Fig. 2:** A typical example of the auto-correlation (AC) analysis on θ/δ and R(t) in a WT mouse
736 (WT #1) in a range of time shift from 0 to 6 hr in the fed ad libitum (Fed) and FD conditions.
737 AC above and below horizontal lines indicate significantly positive and negative correlations at
738 P=0.001, respectively. The broken vertical lines indicate the first peak of θ/δ and a peak of R(t)
739 that appeared at the closest time to that of θ/δ. An amplitude was determined by subtracting a
740 valley from the peak values. Significant level of the AC at peak, the peak time, and the
741 amplitude were used as the criteria to judge whether mice showed significant periodic waves in
742 both θ/δ and R(t) (see text for further details). The results are summarized in **Table 2**.
743
744 **Fig. 3:** Durations of food-seeking, eating and other behaviors for the first 2 hr of the dark phase
745 (i.e., 19:00 to 21:00) in the Fed, FD, and refed (RF) conditions in WT and V1a receptor knockout
746 (V1a KO) mice (**A**), and after local infusion of vehicle control (CNT) or a V1a receptor
747 antagonist (V1a BLK) into the nucleus tractus solitarii of wild-type mice (**B**). The mean and
748 SE bars are presented for 10 WT and 9 V1a KO mice and for 9 CNT and 10 V1a BLK mice.
749 Significant differences from the Fed condition, ** $P<0.01$ and *** $P<0.001$.
750
751 **Fig. 4:** Average change from the Fed to FD condition (Δ) in the cross-correlation between θ/δ
752 and R(t) for the first 6 hr of the dark phase are presented as the mean and SE bars for 10 WT and
753 9 V1a KO mice (**A**) and for 9 CNT and 10 V1a BLK mice (**B**). Similarly, Δfood-seeking
754 behavior for the first 2 hr of the dark phase are presented for 10 WT and 9 V1a KO mice (**C**) and

for 9 CNT and 10 V1a BLK mice (**D**). Significant differences from the Fed condition, * P<0.05, ** P<0.01, and *** P<0.001. # Significant differences between the groups at P<0.05. § Average values after z transformation.

Fig. 1


WT #1
θ/δ
Moving Average
1.5
1.0
0.5
R(t)
Moving Average§
1
0
-1
Raw data
(P=0.05)
(P=0.05)
ΔHR/ΔMAP, beats/min/mmHg
30
0
-30
HR, beats/min
800
300
MAP, mmHg
150
90
Activity, counts/30 sec
30
0
0
6
12
Time, hr

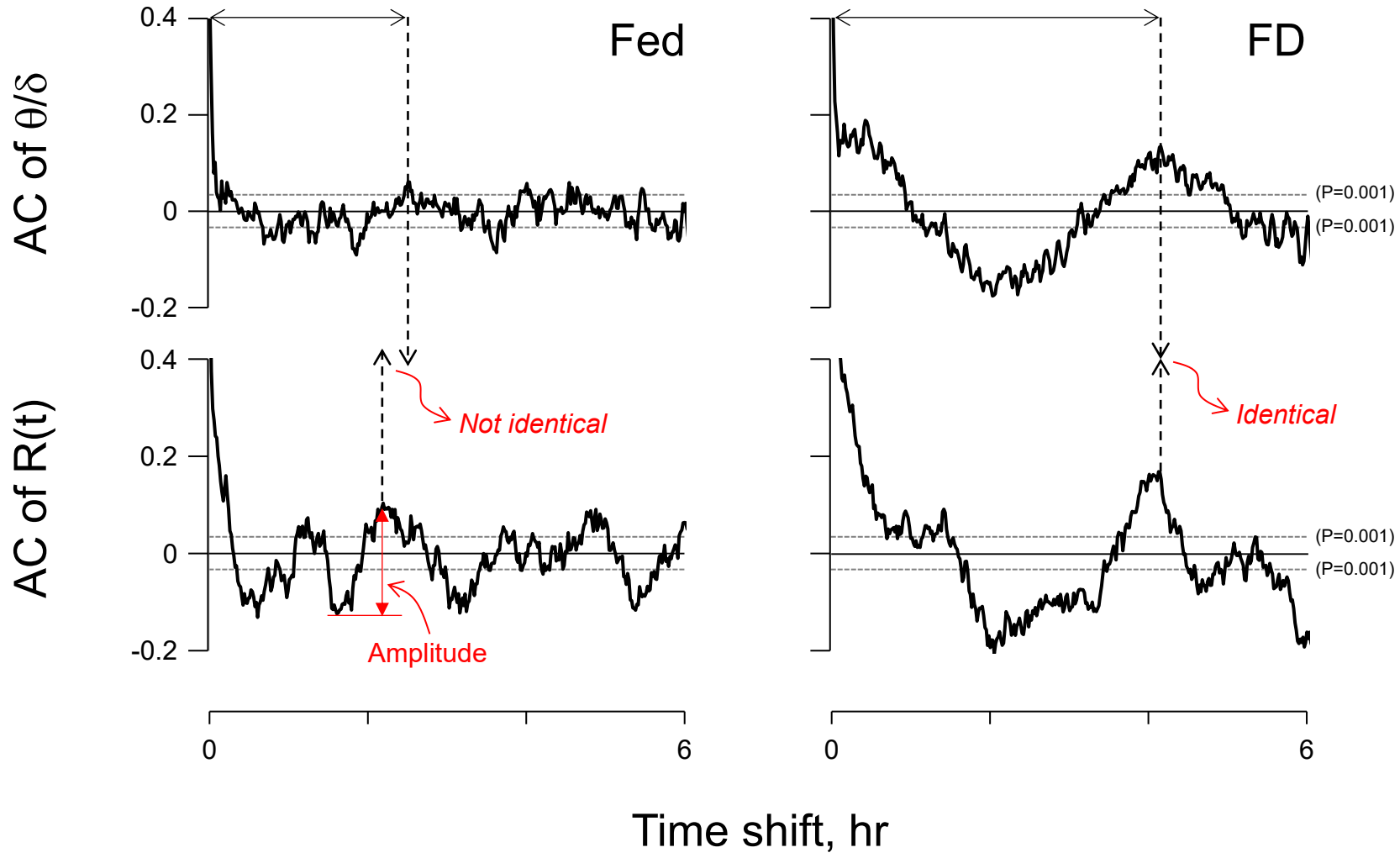
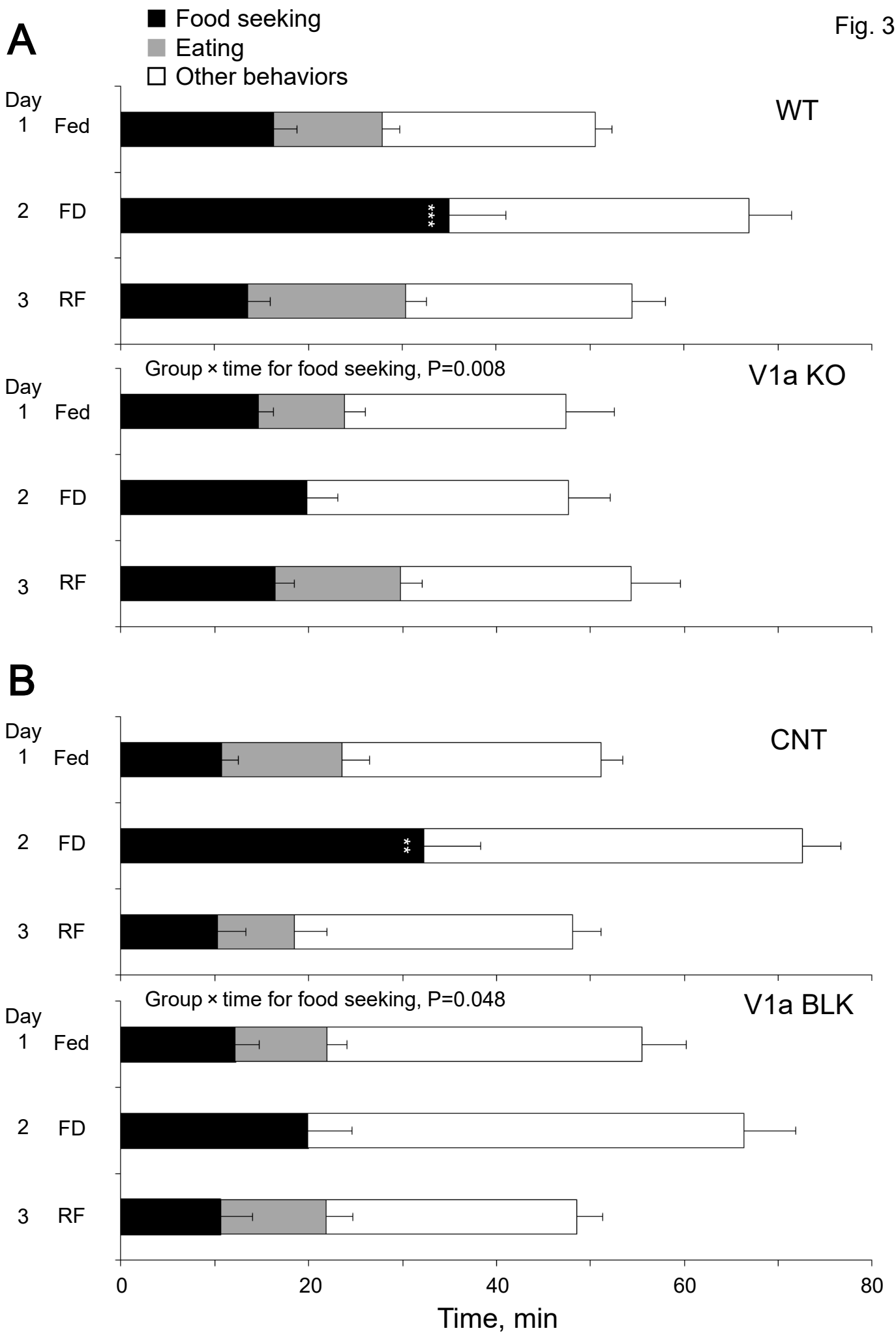

WT #1
Fed
FD
AC of θ/δ
AC of R(t)
0.4
0.2
0
-0.2
(P=0.001)
(P=0.001)
Not identical
Identical
Amplitude
0
6
Time shift, hr

Fig. 3


A
Food seeking
Eating
Other behaviors
Day
1 Fed
2 FD
3 RF
WT
***
Group × time for food seeking, P=0.008
V1a KO
B
CNT
**
Group × time for food seeking, P=0.048
V1a BLK
0
20
40
60
80
Time, min

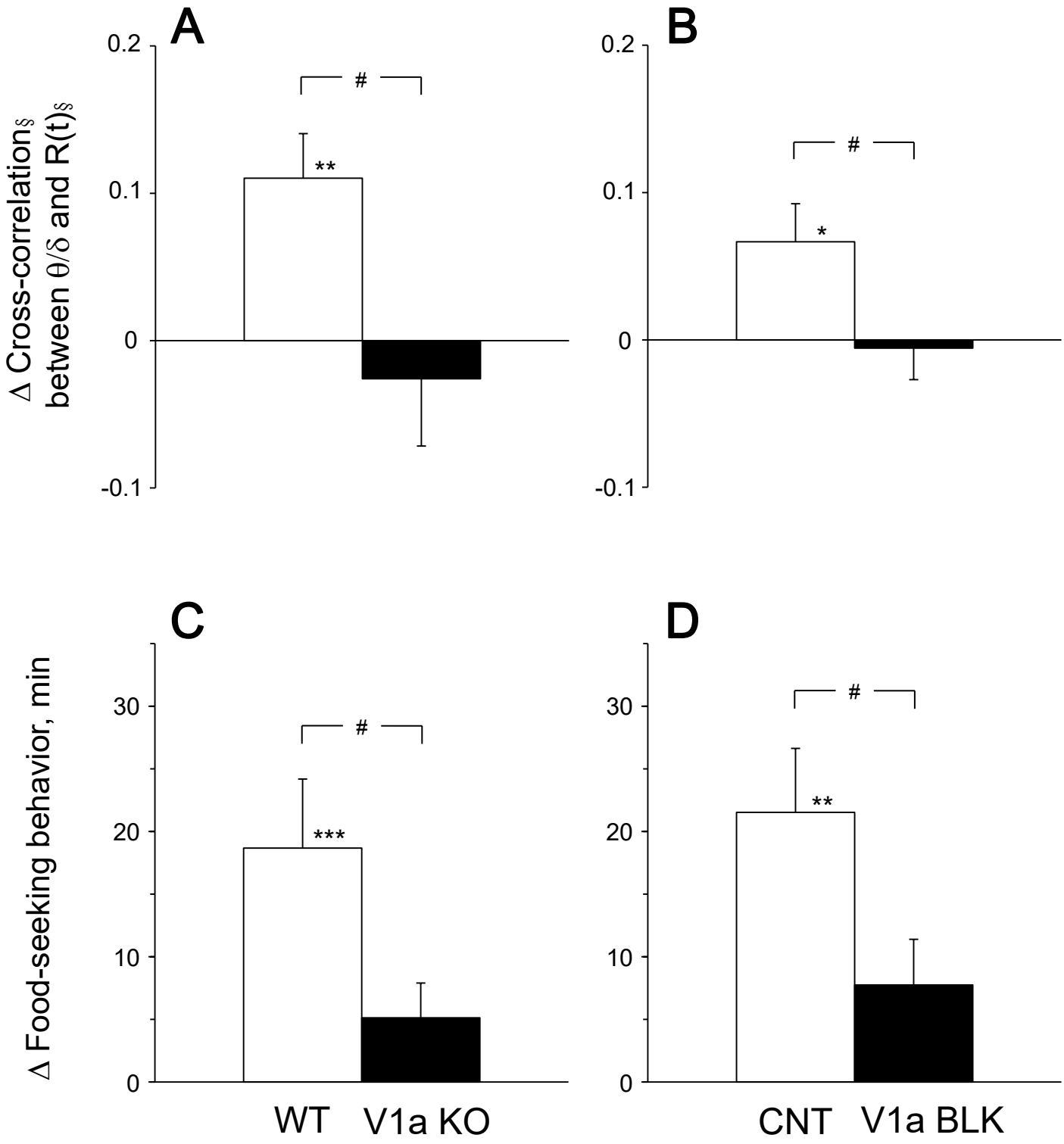

A
B
C
D
Δ Cross-correlation§ between θ/δ and R(t)§
Δ Food-seeking behavior, min
0.2
0.1
0
-0.1
30
20
10
0
#
**
*
***
WT
V1a KO
CNT
V1a BLK

Table 1. θ/δ, R(t) HR, MAP, and activity counts in the fed ad libitum (Fed), food-deprived (FD), and refed (RF) conditions for the first 6 hr of the dark phase

	WT (n=10)			V1a KO (n=9)			
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	# Time x Group
	Fed	FD	RF	Fed	FD	RF	P value
θ/δ	0.68±0.03	0.67±0.03	0.65±0.04	0.81±0.03†	0.79±0.05†	0.82±0.04††	NS
R(t)§	-0.29±0.03	-0.32±0.03***	-0.25±0.03	-0.34±0.04	-0.41±0.03***	-0.19±0.01***	NS
HR, beats/min	494±13	461±14**	495±9	605±13†††	574±18**†††	575±12*†††	NS
MAP, mmHg	115±2	114±2	115±3	115±2	112±2	114±2	NS
Activity, counts/30 sec	2.0±0.3	2.7±0.5	2.3±0.4	2.5±0.4	2.9±0.4	2.2±0.2	NS
	CNT (n=9)			V1a BLK (n=10)			
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	# Time x Group
	Fed	FD	RF	Fed	FD	RF	P value
θ/δ	0.70±0.02	0.69±0.02	0.67±0.02***	0.75±0.04	0.75±0.04	0.73±0.04***	NS
R(t)§	-0.24±0.01	-0.29±0.01***	-0.22±0.02	-0.24±0.01	-0.30±0.02***	-0.22±0.01	NS
HR, beats/min	514±9	488±6**	510±9	513±10	485±15**	499±9	NS
MAP, mmHg	115±3	114±2	113±3	117±2	114±1*	114±2*	NS
Activity, counts/30 sec	2.2±0.2	3.2±0.3***	2.5±0.2	2.0±0.2	3.3±0.4***	2.3±0.4	NS

Values are the mean ± SE. WT, wild-type control mice; V1a KO, V1a receptor knockout mice; CNT, wild-type mice treated with vehicle control in the nucleus tractus solitarii (NTS); V1a BLK, wild-type mice treated with V1a receptor blockade in the NTS; θ/δ, power density ratio of θ to δ wave band on electroencephalogram; R(t), cross-correlation function between ΔHR and ΔMAP; NS, not significant. § Values were averaged after z transformation. Significant differences from the Fed condition, * P<0.05, ** P<0.01, and *** P<0.001. Significant differences from WT mice, † P<0.05, †† P<0.01, and ††† P<0.001. # Interactive effects of time (day 1 vs day 2) x group.

Table 2. The number of mice showing synchronized periodic waves in θ/δ and R(t) by auto-correlation analysis in the Fed and FD conditions for 12 hr of the dark phase

	WT			V1a KO		
	Day 1 Fed	Day 2 FD	Significant periodic waves only in FD	Day 1 Fed	Day 2 FD	Significant periodic waves only in FD
§The number of mice showing synchronized periodic waves in θ/δ and R(t)	1/10 (8)	9/10*** (1**)	8/10	2/9 (7)	0/9††† (8†††)	0/9†††

	CNT			V1a BLK		
	Day 1 Fed	Day 2 FD	Significant periodic waves only in FD	Day 1 Fed	Day 2 FD	Significant periodic waves only in FD
§The number of mice showing synchronized periodic waves in θ/δ and R(t)	0/9 (7)	9/9*** (0**)	9/9	2/10 (6)	1/10††† (8†††)	0/10†††

§ To assess any change in the linkage between θ/δ and R(t) by FD in all groups of mice, we determined the number of mice in the Fed and FD conditions during the dark phase meeting three criteria in both θ/δ and R(t): 1) significant peaks of auto-correlation function at $P<0.001$ in a range of time shift from 0 to 6 hr; 2) its amplitude (a difference between peak and valley values) >0.085 which was a minimal value of 2 SD of auto-correlation function after z transformation in the FD condition in all WT and CNT mice; and 3) a significant peak of R(t) meeting the first criterion occurred within ±7 min from the first peak of θ/δ, of which the range was the closest to but out of 1 SD of the difference in the peak time between θ/δ and R(t) in the FD condition in all WT and CNT mice. The values enclosed in the parentheses indicate the number of mice meeting criteria 1) and 2) but not meeting criterion 3). *** Significant differences from the Fed condition, $P<0.001$. ††† Significant differences from the WT or CNT mice, $P<0.001$. Other abbreviations are the same as in **Table 1**.

Table 3. Cross-correlation between θ/δ and R(t) in the Fed, FD, and RF conditions for the first 6 hr of the dark phase

	WT (n=10)			V1a KO (n=9)			
	Day 1 Fed	Day 2 FD	Day 3 RF	Day 1 Fed	Day 2 FD	Day 3 RF	# Time x Group P value
Cross-correlation§ between θ/δ and R(t)§	0.21±0.04	0.32±0.05**	0.27±0.06	0.24±0.05	0.21±0.05	0.28±0.04	0.021
	CNT (n=9)			V1a BLK (n=10)			
	Day 1 Fed	Day 2 FD	Day 3 RF	Day 1 Fed	Day 2 FD	Day 3 RF	# Time x Group P value
Cross-correlation§ between θ/δ and R(t)§	0.14±0.02	0.20±0.03*	0.18±0.03	0.19±0.04	0.19±0.05	0.18±0.03	0.045

Values are the mean ± SE. § Values were averaged after z transformation. Significant differences from the Fed condition, * P<0.05 and ** P<0.01. # Interactive effects of time (day 1 vs day 2) x group. Other abbreviations are the same as in **Table 1**.

Role of linkage between cerebral activity and baroreflex control of heart rate via central vasopressin V1a receptors in food-deprived mice

METHODS

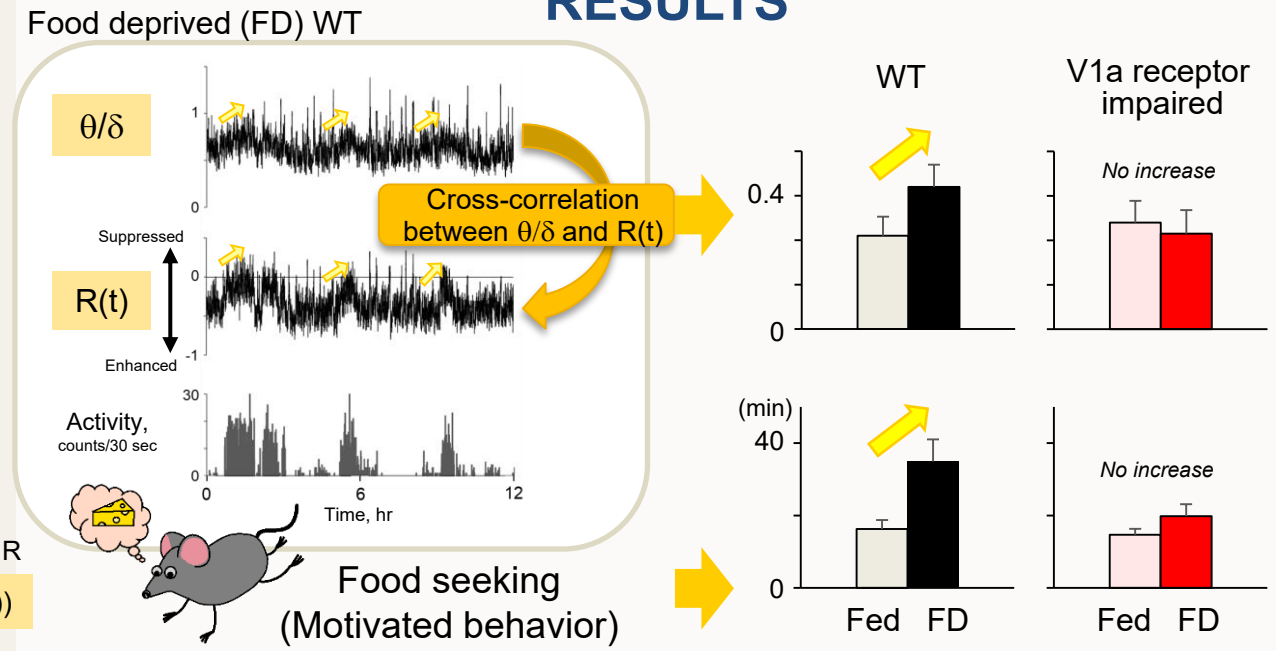
Animals

- Wild-type (WT) mice
- V1a receptor knockout mice
- Wild-type mice locally infused with a V1a receptor antagonist into the nucleus tractus solitarii

Measurements

1) Electroencephalogram
   → Ratio of θ to δ wave
   An index of cerebral activity (θ/δ)
2) Arterial pressure (AP)
3) Heart rate (HR)
   → Cross-correlation between changes in AP & HR
   An index of baroreflex control of HR (R(t))
4) Activity: Locomotion sensors
5) Animals' behavior: CCD camera

RESULTS



The enhancements by FD from the fed ad libitum (Fed) condition were abolished in V1a receptor-impaired mice.

CONCLUSION The suppression of baroreflex control of HR linked with cerebral activation via V1a receptors might play an important role at the onset of motivated behaviors, such as food seeking induced by FD, by enhancing pressor response.