Available online at www.sciencedirect.com
SCIENCE DIRECT®
Brain Research 995 (2004) 97–108
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Single-unit activity of paraventricular nucleus neurons in response to intero- and exteroceptive stressors in conscious, freely moving rats

Shoichi Watanabe[a], Takato Kunitake[a], Kazuo Kato[a], Chun-Ping Chu[a], Hiroyuki Nakao[b], De-Lai Qiu[b], Hiroshi Kannan[a,*]

[a] *Department of First Physiology, Miyazaki Medical College, 5200 Kihara, Kiyotake-cho, Miyazaki-gun, Miyazaki 889-1692, Japan*
[b] *Department of Public Health, Miyazaki Medical College, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan*

Accepted 25 September 2003

Abstract

Extracellular recordings of 114 neurons in the hypothalamic paraventricular nucleus (PVN) of conscious, freely moving male rats were performed using a movable electrode system. Single-unit activities were examined for their spontaneous firing patterns and responses to intero- and exteroceptive stressors, including disturbance in arterial blood pressure, water deprivation, air-jet stimulation, and systemic administration of cholecystokinin-8 (CCK). PVN neurons were assigned to one of two groups on the basis of their spontaneous firing patterns: phasic ($n=29$) and non-phasic ($n=85$). Intravenous (i.v.) administration of phenylephrine (8 μg/kg) resulted in the inhibition of a greater percentage of phasic-type (88.9%; 24/27) than non-phasic-type neurons (14.9%; 11/74). Most phasic-type neurons showed excitation in response to i.v. administration of sodium nitroprusside (20 μg/kg, 66.7%; 18/27) and water deprivation (15 h, 77.8%; 7/9) when compared to non-phasic-type neurons. Conversely, a greater number of non-phasic-type neurons showed excitation in response to air-jet stimulation (5 l/min, 10 s, 29.0%; 20/69) and to i.v. administration of CCK (5 μg/kg, 24.5%; 11/45) when compared to phasic-type neurons. However, most non-phasic-type neurons that demonstrated excitation in response to i.v. administration of CCK (88.9%; 8/9) did not respond to air-jet stimulation. The present study indicated that phasically firing neurons recorded from the PVN in conscious, freely moving rats are putative vasopressin-secreting neurons on the basis of their responses to intero- and exteroceptive stressors. These data contribute to our understanding of local neural mechanisms within the PVN that are responsible for stress responses in conscious rats.

Theme: Neural basis of behavior
Topic: Stress

Keywords: Extracellular recording; Paraventricular nucleus; Phasic-type neuron; Stressor

1. Introduction

The hypothalamic paraventricular nucleus (PVN), located along the third ventricle, integrates autonomic and neuroendocrine activity [50,60,61] and functions to maintain physiological homeostasis during the application of intero- and exteroceptive stressors [22,42]. A distinction has been proposed between the pathways responding to systemic or homeostatic stressors, which have been termed interoceptive, vs. those that respond to processive or emotional stressors, referred to as exteroceptive [14,42]. The PVN neurons, which receive various stress-related signals, are involved in the control of autonomic outflow and the hypothalamo-pituitary-adrenal (HPA) axis [62]. The majority of the corticotropin-releasing hormone (CRH)-secreting neurons that project to the median eminence (ME) originate in the PVN [30]. Stressors that activate the HPA axis do so in large part by activating parvocellular CRH neurons. However, the mechanisms by which various stressors activate the PVN neurons remain unclear.

Previous electrophysiological studies of the PVN performed in anesthetized animals [9,39,57] have demonstrated characteristic responses in the PVN subset to various stressors [24,53,59]. In particular, arginine vasopressin

* Corresponding author. Tel.: +81-985-85-0870; fax: +81-985-85-5805.
E-mail address: kannanh@post.miyazaki-med.ac.jp (H. Kannan).

0006-8993/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2003.09.058

(AVP)-secreting neurons demonstrated a phasic firing pattern [18,38,39,59]. AVP secretion in response to hyperosmolality and hypovolemia is known to preserve plasma and fluid volume [5,12,19,38]. However, autonomic and neuroendocrine responses are dramatically influenced by surgery/anesthetics, and opposite results may be produced when comparing studies in conscious vs. anesthetized animals. Further, baroreflex impairment, sympathoadrenal activation, and neurohumoral mechanisms are highly variable in the anesthetized state and difficult to quantify [26,46,63]. For example, electrical stimulation of the PVN produced excitatory responses in renal sympathetic nerve activity in unanesthetized rats but not in anesthetized rats [26]. In addition, pentobarbital sodium, chloralose, and urethane, which are commonly used in animal experiments, have different effects on noradrenergic activity in the PVN region [43].

The goal of the present study was to investigate the heterogeneity in the PVN response to intero- and exteroceptive stressors under freely "physiologic" conditions. While a phasic firing pattern is present in the hypothalamus of conscious rabbits and monkeys [2,48,56], the response to intero- and exteroceptive stressors has not been characterized. Therefore, we recorded single-unit activity of PVN neurons in conscious, freely moving rats in response to various stressors, including disturbance in blood pressure (BP) [5,13,18,19,24,38,53], water deprivation [23,40,44], air-jet stimulation [33,52], and intravenous (i.v.) administration of cholesystokinin-8 (CCK) [7,55].

2. Materials and methods

2.1. Subjects

Twelve adult male Wistar rats, weighing 350–450 g at arrival, were housed individually under a constant temperature of 23 ± 1 °C in a light-controlled room (light on at 0700–1900 h) with rat chow and tap water available ad libitum except as required by the experiments (see below). Experimental protocols were approved by the Committee on Animal Care of Miyazaki Medical College.

2.2. Surgical preparations

After anesthesia with intraperitoneal administration of pentobarbital sodium (50 mg/kg), an SP-31 polyethylene tubing heat-coupled to an SP-50 catheter was inserted into the right femoral artery for BP and heart rate (HR) measurement, and a PE-50 catheter was inserted into the right femoral vein for the administration of drugs. Both catheters were tunneled under the skin to exit at the nape of the neck. The arterial catheter, filled with heparinized (10 U/ml) saline solution, was connected to a Statham pressure transducer (Gould, Saddle Brook, NJ) to monitor BP, and the venous catheter was sealed. BP and HR were monitored with a Unique-Acquisition-System (Unique Medical, Tokyo, Japan) through a cardiotachometer (model 1321; San-Ei, Tokyo, Japan). Adequate depth of anesthesia was maintained by hourly administration of additional pentobarbital sodium (10 mg/kg, i.v.). Body temperature was maintained at 37 °C with a heating pad.

Each animal was placed in the prone position, and the head was fixed in a stereotaxic instrument (Narishige, Tokyo, Japan). Craniotomy was performed for the implantation of the recording electrodes into the hypothalamus. The tip of the stainless steel outer cannula (0.9 mm diameter) with eight electrodes was positioned at a depth of 5.5 mm from the cortex surface and 1.9 mm above the right PVN with a stereotaxic manipulator through a burr hole located 2.1 mm posterior and 0.35 mm right lateral to the bregma. The microdrive component was fixed to the skull via anchoring screws and dental cement.

After the surgical operation, each animal was placed in a computer-controlled cylindrical metabolic cage (28 cm in diameter × 28 cm in height) to habituate to the experimental environment. The arterial catheter was connected to an infusion pump that supplied 2 μl/min of heparinized saline (10 U/ml) throughout the period of recording. In addition, for the application of air-jet, a silicon tube (model 100-2 N, Kaneka medix, Osaka, Japan) was fixed to the front of the microdrive, 1.0 cm above the mid-forehead, and connected to an air compressor (model theta 6000, Nisso, Tokyo, Japan).

2.3. Extracellular unit recording

Recording electrodes were constructed from Nichrome wire (25 μm diameter, model #7615, A-M Systems, WA, USA) coated with multiple layers of epoxy resin (model #6001, The EPOXYLITE, CA, USA). The diameter of the insulated electrode was 50 μm, and the impedance was between 1.3 and 1.5 MΩ at 1000 Hz. Eight electrodes were fixed into the inner guide tube (0.6 mm diameter), with the tips protruding ~ 5.0 mm in length. The tip of the outer tube and the electrodes were trued with the edges. Distances between each electrode on the level surface were approximately 200–300 μm. With this apparatus, the motion is transferred via the carrier plate to produce a dorso-ventral movement of the sliding inner guide tube through which eight electrodes are affixed. The forward-reverse drive mechanism of the microdrive is controlled via clockwise or counterclockwise rotation with a manual screwdriver technique (one turn equals 400 μm). Thus, this microdrive permitted discrete stable positioning of the electrode tip in the brain.

Three days after surgical operation, the tips of the electrodes were lowered to a depth 1.6 mm below the tip of the outer cannula. Seven days after surgical operation, the electrodes were gradually lowered, and single-unit extracellular recordings were obtained.

2.4. Experimental protocols

2.4.1. Spontaneous firing and cardiovascular data before stimulation

The region of the PVN was systematically explored for spontaneous extracellularly recorded active single units of 12 rats. A minimum of 5 min of baseline activity was used for evaluation of a spontaneous firing rate and pattern. Neurons recorded in the PVN were divided into one of two groups on the basis of their spontaneous firing patterns: phasic and non-phasic. Phasic-type neurons were characterized by alternating periods of silence ('off' phase) and activity ('on' phase) (Fig. 2), as shown in previous studies [38,39,57]. The spontaneous firing rate averages were compared between the two groups.

2.4.2. Baroreceptor activation

Phenylephrine (PE, Kowa, Nagoya, Japan) was dissolved in 0.15 M NaCl solution and injected into 12 rats as an i.v. bolus (8 μg/kg) at least twice with, at least, a 5-min interval. Successful baroreceptor activation was confirmed by observations of decreased HR in response to an increase in BP. The cardiovascular and neural responses were monitored until cardiovascular responses returned to baseline (at least 5 min).

2.4.3. Baroreceptor unloading

Sodium nitroprusside (SNP, Sigma, St. Louis, MO, USA) was dissolved in a 0.15-M NaCl solution and injected into 12 rats as an i.v. bolus (20 μg/kg) at least twice with, at least, 5-min intervals. Successful baroreceptor unloading was confirmed by observations of increased HR in response to a decrease in BP. The cardiovascular and neural responses were monitored until cardiovascular responses returned to baseline (at least 5 min).

2.4.4. Water deprivation

For eight rats, a water bottle was removed from the animal's cage at 1800 h, and 15-h later, firing records and cardiovascular data were obtained for 20 min, after which the water bottle was returned to the cage. Food was available ad libitum during the water deprivation period. After the recordings were complete, the water bottle was returned to the cage.

2.4.5. Air-jet stimulation

Air-jet stimulation to the mid-forehead of eight rats was performed via three successive 10-s-long air-jet streams (5 l/min) separated by 5-min intervals. Successful air stimulation was confirmed by the observation of transient tachycardia [22]. The tests were performed at least three times to verify reproducibility. The cardiovascular and neural responses were monitored until cardiovascular responses returned to baseline (at least 1 min).

2.4.6. Systemic administration of CCK

Cholecystokinin-8 (CCK, Peptide Institute, Osaka, Japan) was dissolved in a 0.15-M NaCl solution and injected into six rats as an i.v. bolus (5 μg/kg). The CCK dose was based on previous reports that characterized the concentrations required for cardiovascular response [53], and CCK was administered once a day. The cardiovascular and neural responses were monitored until cardiovascular responses returned to baseline (at least 15 min).

The protocol of the experiments is explained in the following text. First, the order followed included air-jet stimulation, baroreceptor activation, and unloading. Next, CCK was administered, and then water deprivation commenced. The experiments were separated by, at least, 5-min intervals. A rest period of over 2 h was scheduled after administration of CCK and water deprivation. After five separate experiments had been performed, the electrodes were inserted deeper than 50 μm to detect another neuron.

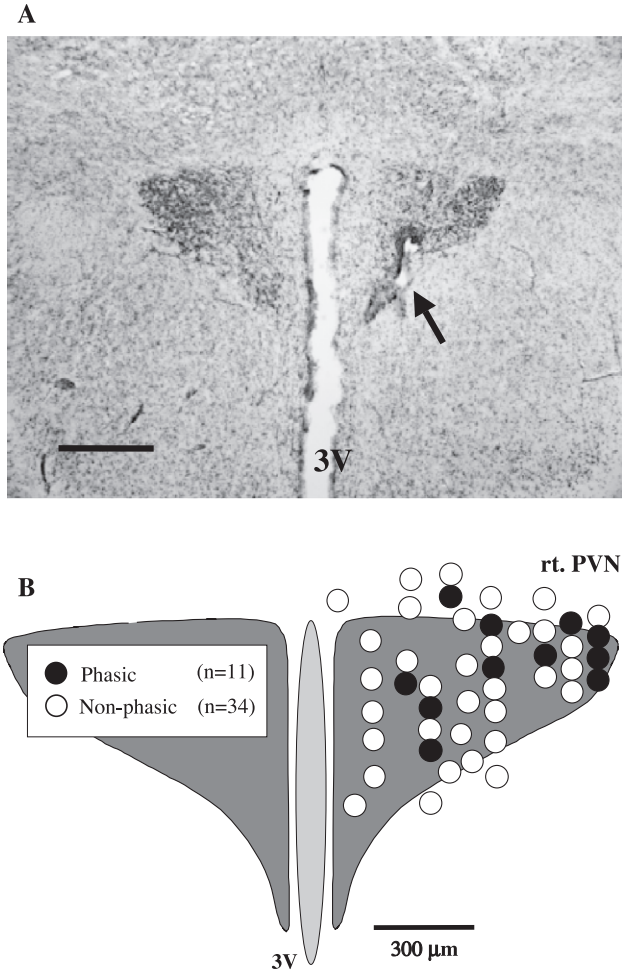

Fig. 1. (A) A photomicrograph of a coronal PVN section for reconstruction. The arrow indicates an electrical lesion of the PVN at the tip of the central electrode. 3V, third ventricle. Scale bar = 500 μm. (B) Schematic drawings of the PVN show the locations of the neurons tested for their responses to stressors. A closed circle indicates a phasic-type neuron ($n = 11$). An open circle indicates a non-phasic-type neuron ($n = 34$).

2.5. Data acquisition and analysis

Electrode signals were routed through AC preamplifiers (model VC-9; Nihon-Kohden, Tokyo, Japan) to a Unique-Acquisition-System (Unique Medical). An active filter (pass band 100–3000 Hz) was used to eliminate low- and high-frequency signals. This system was used for digitized analog signal acquisition, signal display, and online analysis on the software oscilloscopes as well as for storage of the signals on a digital videodisc. The sampling frequency was 10 kHz. An advantage of this system is the simultaneous display and storage of data related to BP, HR, and neural activity. The signals were monitored continuously on software oscilloscopes and a standard oscilloscope (model VC-9; Nihon-Kohden) with an audio monitor. Off-line analysis of the recorded signals was performed using the same processing software. Wave sorting was performed for each recorded file by setting a threshold for passing areas defined as voluntary. This ensured good quality of separation of a single unit from other units and artifacts. The electrical activity of a neuron was expressed in terms of the firing rate (action potential/s), and peristimulus time histograms (2-s bin) of evoked responses from the PVN neurons were generated. Subsequent changes in firing rates after the application of a stimulus were expressed as a percentage of the control firing rate. A mean bin height of at least 40% above or below the mean baseline firing rate (calculated from the preceding 300 s) was accepted as a significant response.

The individual data were expressed as means ± S.E.M. The averages of the spontaneous firing rate were compared between two groups using the Student's *t*-test. The averages of BP and HR before and after the various stimuli were compared by the paired *t*-test. Differences in the proportions of the responses to each stressor between the phasic type and non-phasic type were evaluated using Fisher's exact probability test. All statistical significance was defined at the level of $P = 0.05$.

2.6. Histology

At the end of each experiment, each rat was killed with an overdose injection of pentobarbital sodium. At least one site of the central electrode was marked with a small electrolytic lesion made by the application of 20 μA of direct current for 90 s. The brain was removed and fixed in 10% neutral-buffered formalin for 2 days. Coronal sections (40 μm) were stained with Cresyl violet. Histological verification of the locations of the electrode tips was performed by light microscopy for reconstruction according to the rat brain atlas of Paxinos and Watson [37].

3. Results

3.1. Spontaneous unit activity of the PVN under resting conditions

Successful extracellular single-unit recordings were obtained from 114 spontaneously active PVN neurons, as confirmed by histological reconstruction. Of these, 45 units were obtained from 12 electrodes to which current was supplied (Fig. 1A). Phasic-type neurons ($n = 11$) were recognized largely in the lateral PVN (Fig. 1B), while non-phasic-type neurons ($n = 34$) were located diffusely throughout the PVN. Sixty-nine residual units were obtained from other electrodes within the PVN region.

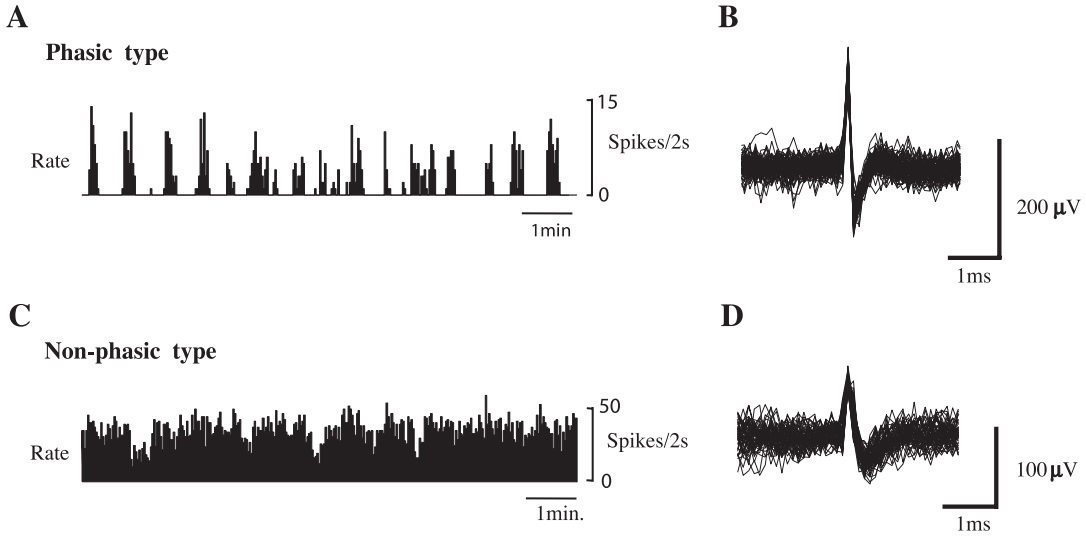

Fig. 2. Representative example of the spontaneous firing pattern in the PVN neurons. (A) Time histogram (2-s bin size) showing the spontaneous firing pattern of a phasic-type neuron. (B) Analog wave forms detected in the neuron of panel A. (C) Time histogram showing the spontaneous firing pattern of a non-phasic-type neuron. (D) Analog wave forms detected in the neuron of panel C.

All neurons were characterized as phasic ($n=29$, 25.4%) or non-phasic type ($n=85$, 74.6%). Fig. 2 shows representative records of the phasic and non-phasic firing patterns of the PVN neurons. The averages of the spontaneous firing rate were significantly higher ($P<0.001$) in non-phasic-type neurons (4.56 ± 0.50 spikes/s) than in phasic-type neurons (1.58 ± 0.16 spikes/s). In phasic-type neurons, the active phase duration was 23.9 ± 2.1 s, and the silent phase duration was 15.0 ± 2.1 s. The mean intraburst firing rate was 2.43 ± 0.31 spikes/s. The mean arterial blood pressure (MAP) was 91.5 ± 0.7 mm Hg ($n=114$), and the HR was 305.5 ± 3.9 bpm ($n=114$) under resting conditions.

3.2. PVN neuron response to various stressors

3.2.1. Baroreceptor activation

One hundred and one neurons, histologically verified within the PVN region, were tested in animals that underwent PE-induced baroreceptor activation (Table 1, Fig. 3). Compared to pre-stimulation, the average MAP change was $+55.9 \pm 0.9$ mm Hg ($P<0.001$), and the average HR change was -153 ± 6.0 bpm ($P<0.001$). In all, 35 neurons (34.6%) were inhibited (Fig. 3A), 8 (8.0%) were excited (Fig. 3C), and 58 (57.4%) were non-responsive. A significantly greater percentage (88.9%; 24/27) of phasic-type neurons were inhibited in response to baroreceptor activation compared to non-phasic-type neurons, while a greater percentage (75.7%, 56/74) of non-phasic-type neurons showed no response to baroreceptor activation when compared to phasic-type neurons ($P<0.001$). There was no difference between the groups in terms of changes in MAP or HR.

3.2.2. Baroreceptor unloading

One hundred and one neurons were tested from animals that underwent SNP-induced baroreceptor unloading (Table 1, Fig. 4). Compared to pre-stimulation, the average MAP change was -34.7 ± 0.6 mm Hg ($P<0.001$), and the average HR change was $+111.7 \pm 2.7$ bpm ($P<0.001$). In all, 23 neurons (22.8%) were excited (Fig. 4A), 10 (9.9%) were inhibited (Fig. 4C), and 68 (67.3%) were non-responsive. A significantly greater percentage (66.7%; 18/27) of phasic-type neurons were excited by baroreceptor unloading when compared to non-phasic-type neurons ($P<0.001$). Typically, baroreceptor unloading resulted in an increased duration of the active phase (Fig. 4A). A significantly greater percentage (81.1%, 60/74) of non-phasic-type neurons showed no response to baroreceptor unloading when compared to phasic-type neurons ($P<0.001$). There was no difference between the groups in terms of changes in MAP or HR.

3.2.3. Water deprivation

Thirty neurons were tested in animals that underwent water deprivation for 15 h (Table 1, Fig. 5). Compared to pre-water deprivation, the average MAP change was $+1.4 \pm 1.2$ mm Hg ($P>0.05$), and the average HR change was -19.4 ± 4.0 bpm ($P<0.001$). In all, 13 neurons (43.3%) were excited (Fig. 5A,C), 9 (30.0%) were inhibited, and 8 (26.7%) were non-responsive. A significantly greater percentage (77.8%; 7/9) of phasic-type neurons showed an excited response to water deprivation when compared with

Table 1
PVN neuron response to various stressors

	Baroreceptor activation		Baroreceptor unloading	
	Phasic	Non-phasic	Phasic	Non-phasic
Excitation	1 (3.7)	7 (9.4)	18 (66.7)	5 (6.7)
Inhibition	24 (88.9)	11 (14.9)	1 (3.7)	9 (12.2)
No response	2 (7.4)	56 (75.7)	8 (29.6)	60 (81.1)
Total	27 (100.0)	74 (100.0)	27 (100.0)	74 (100.0)
P-value*				
Response vs. no response	<0.001		<0.001	
Excitation vs. inhibition	<0.01		<0.01	
Excitation vs. others			<0.001	
Inhibition vs. others	<0.001			

	Water deprivation		Air-jet stimulation	
	Phasic	Phasic	Phasic	Non-phasic
Excitation	7 (77.8)	6 (28.6)	1 (4.2)	20 (29.0)
Inhibition	1 (11.1)	8 (38.1)	1 (4.2)	6 (8.7)
No response	1 (11.1)	7 (33.3)	22 (91.6)	43 (62.3)
Total	9 (100.0)	21 (100.0)	24 (100.0)	69 (100.0)
P-value*				
Response vs. no response			<0.01	
Excitation vs. inhibition				
Excitation vs. others	<0.05		<0.05	
Inhibition vs. others				

	CCK administration	
	Phasic	Phasic
Excitation	0 (0.0)	11 (24.5)
Inhibition	17 (100.0)	23 (51.0)
No response	0 (0.0)	11 (24.5)
Total	17 (100.0)	45 (100.0)
P-value*		
Response vs. no response	<0.05	
Excitation vs. inhibition	<0.01	
Excitation vs. others	<0.05	
Inhibition vs. others	<0.001	

* Differences in the proportions of neural responses to each stressor between phasic-type and non-phasic-type neurons were evaluated using Fisher's exact probability test. The numbers in parentheses indicate percentages.

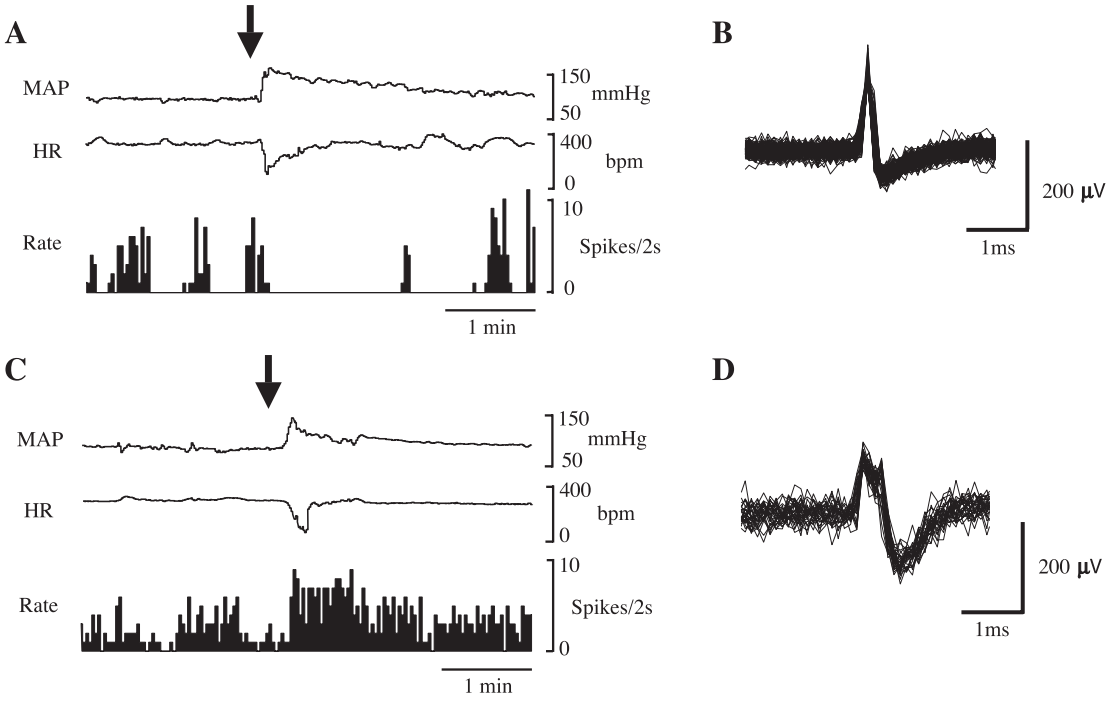

Fig. 3. Representative examples of the PVN neuron response to baroreceptor activation. (A) An intravenous bolus of phenylephrine (PE; 8 μg/kg), which increased mean arterial blood pressure (MAP; top) by 66 mm Hg, inhibited the spontaneous firing rate of this phasic-type neuron (91.7%, bottom). The maximal decrease in heart rate (HR; middle) was 228 bpm. (B) Analog wave forms detected in the neuron of panel A. (C) An intravenous bolus of PE, which increased MAP (top) by 63 mm Hg, increased the spontaneous firing rate of this non-phasic-type neuron (174.8%, bottom). The maximal decrease in HR (middle) was 214 bpm. (D) Analog wave forms detected in the neuron of panel C.

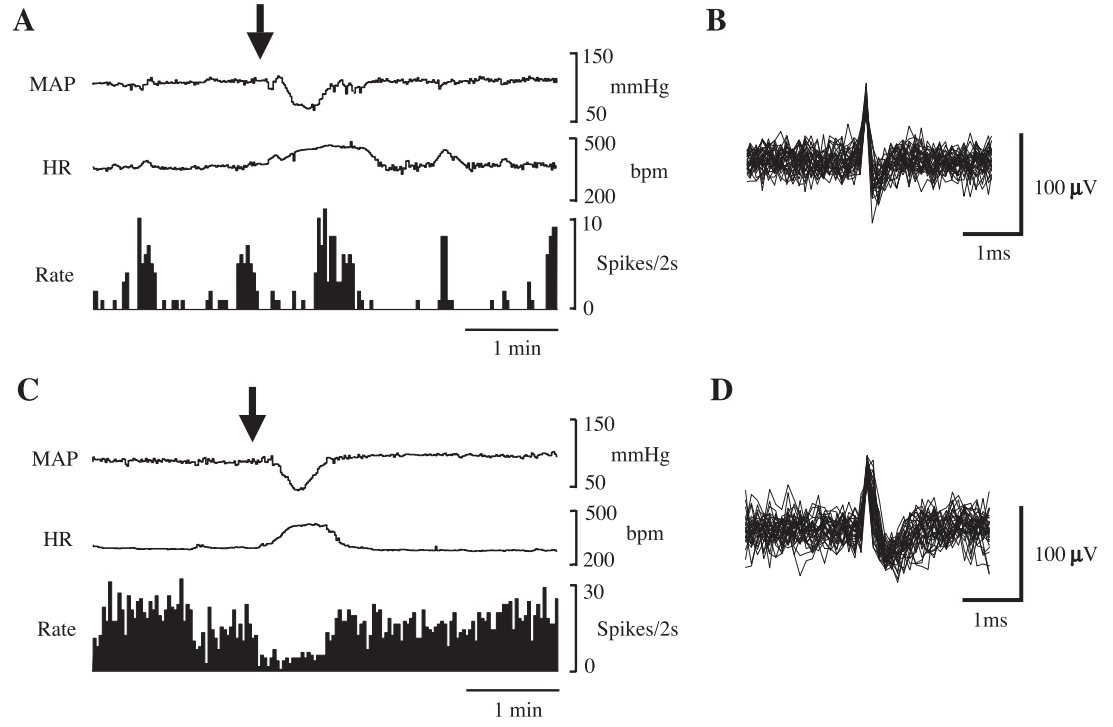

Fig. 4. Representative examples of the PVN neuron response to baroreceptor unloading. (A) An intravenous bolus of sodium nitroprusside (SNP; 20 μg/kg), which decreased MAP (top) by 39 mm Hg, increased the spontaneous firing rate of this phasic-type neuron (51.8%, bottom). The maximal decrease in HR (middle) was 107 bpm. (B) Analog wave forms detected in the neuron of panel A. (C) An intravenous bolus of PE, which decreased MAP (top) by 44 mm Hg, inhibited the spontaneous firing rate of this non-phasic-type neuron (62.6%, bottom). The maximal decrease in HR (middle) was 121 bpm. (D) Analog wave forms detected in the neuron of panel C.

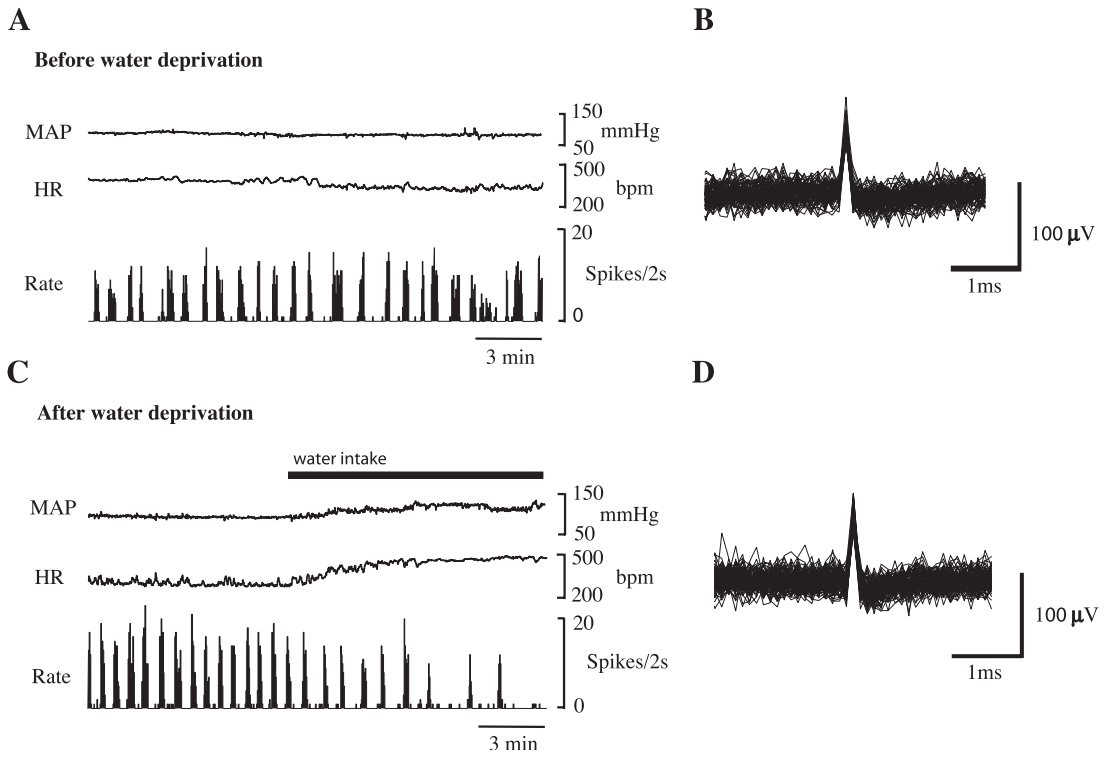

Fig. 5. Representative example of the PVN neuron response to water deprivation. (A) Before water deprivation, the spontaneous firing of this neuron showed a phasic pattern (bottom). MAP (top) and HR (middle) were comparatively stable. (B) Analog wave forms detected in the neuron of panel A. (C) After 15-h water deprivation, the spontaneous firing rate increased by 57.4%. MAP increased slightly (10.2 mm Hg), and HR decreased slightly (15.7 bpm). Increases in MAP and HR and a decrease in the firing rate occurred after reintroduction of drinking water (bold line). (D) Analog wave forms detected in the neuron of panel C.

non-phasic-type neurons ($P<0.05$). In particular, the neurons from these animals primarily displayed an increase in the intraburst firing rate ($P<0.001$, by the paired *t*-test) rather than an increased duration ($P>0.05$, by the paired *t*-test) of the active phase (Fig. 5A,C). In addition, water intake gradually suppressed neural activity (Fig. 5C). There was no difference between the groups in terms of changes in MAP or HR.

3.2.4. Air-jet stimulation

Ninety-three neurons were tested in animals that underwent air-jet stimulation (Table 1, Fig. 6). Compared to pre-stimulation, the average MAP change was $+0.4\pm0.5$ mm Hg ($P>0.05$), and the average HR change was $+20.9\pm1.9$ bpm ($P<0.001$). In all, 21 neurons (22.6%) were excited (Fig. 6A), 7 (7.5%) were inhibited (Fig. 6C), and 65 (69.9%) were non-responsive. A significantly greater percentage (29.0%; 20/69) of non-phasic-type neurons showed an excited response to air-jet stimulation when compared with phasic-type neurons ($P<0.05$). A significantly greater percentage (91.6%, 22/24) of phasic-type neurons showed no response to air-jet stimulation when compared with non-phasic-type neurons ($P<0.01$). There was no difference between the groups in terms of changes in MAP or HR.

3.2.5. Systemic administration of CCK

Sixty-two neurons were tested in animals that underwent systemic administration of CCK (Table 1, Fig. 7). In all cases, animals adopted a freezing posture, which is indicative of anxiety [10,28]. MAP increased immediately after administration of CCK, and HR decreased. Compared to pre-stimulation, the maximal increase in MAP was $+26.9\pm1.0$ mm Hg ($P<0.001$), and the maximal decrease in HR was -64.9 ± 2.8 bpm ($P<0.001$). Subsequently, both MAP and HR progressively returned to baseline values over a period of several minutes. In all, 11 neurons (17.7%) were excited with kinetics resembling changes in MAP (Fig. 7A). Conversely, 40 neurons (64.6%) were inhibited over a similar time-course (Fig. 7C), and 11 neurons (17.7%) were non-responsive. A significantly greater percentage (24.5%; 11/45) of non-phasic-type neurons showed an excited response to the administration of CCK when compared to phasic-type neurons ($P<0.05$). A significantly greater percentage (100.0%; 17/17) of phasic-type neurons showed an inhibited response to the administration of CCK when compared to non-phasic-type neurons ($P<0.001$). Furthermore, a significantly greater percentage (24.5%; 11/45) of non-phasic-type neurons showed no response to the administration of CCK when com-

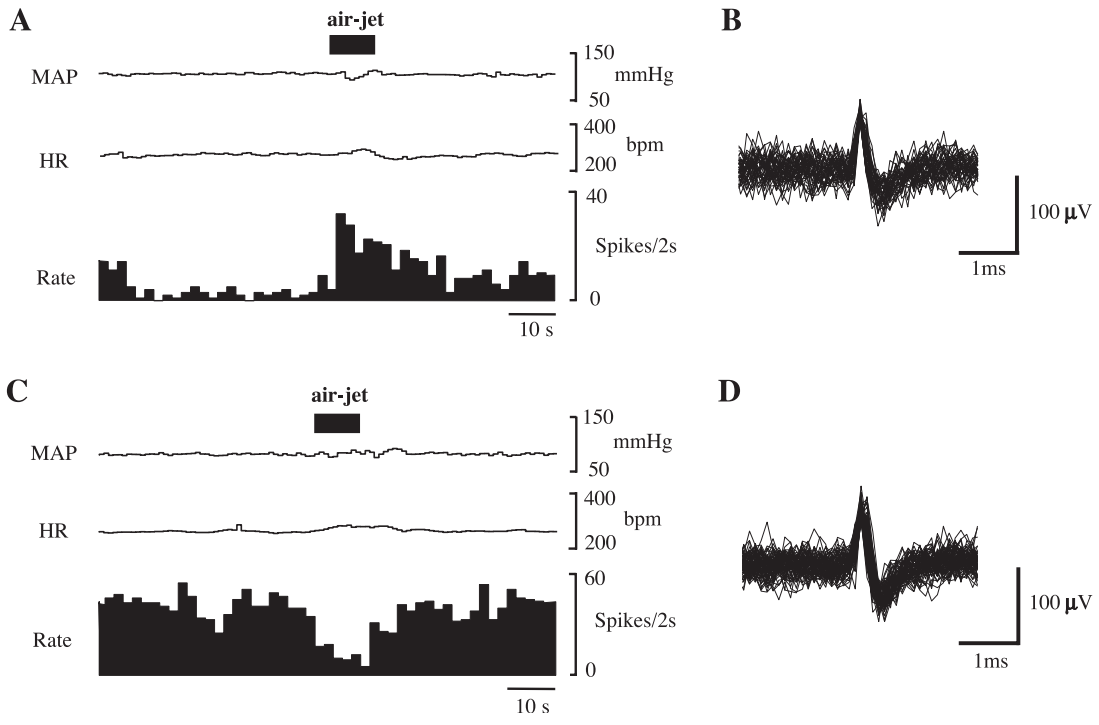

Fig. 6. Representative examples of the PVN neuron response to air-jet stimulation. (A) A 10-s air-jet stimulation (a bold stick line; 5 l/min) increased the spontaneous firing rate of this non-phasic-type neuron (109.5%, bottom). Changes of MAP (top) and HR (middle) were also recognized. (B) Analog wave forms detected in the neuron of panel A. (C) A 10-s air-jet stimulation (5 l/min) inhibited the spontaneous firing rate of this non-phasic-type neuron (65.6%, bottom). Though HR showed transient increase (middle), changes in MAP were variable (top). (D) Analog wave forms detected in the neuron of panel C.

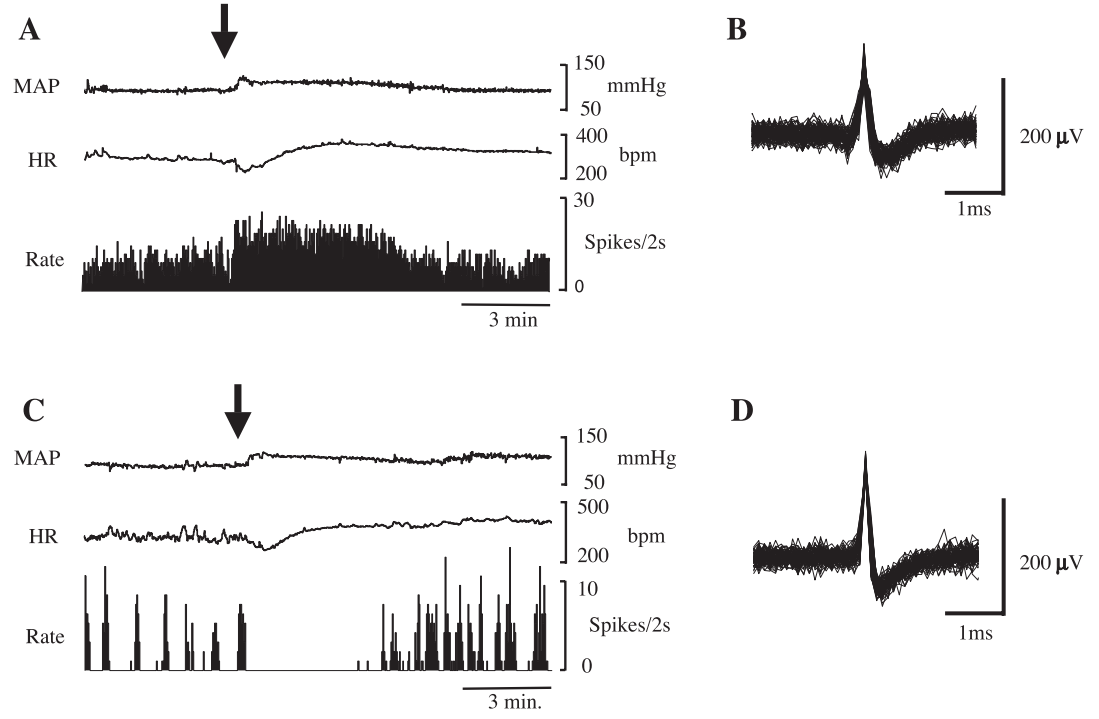

Fig. 7. Representative examples of the PVN neuron response to i.v. administration of CCK. (A) An i.v. bolus of CCK (5 μg/kg), which increased MAP (top) by 33 mm Hg, increased the spontaneous firing rate of this non-phasic-type neuron (76.2%, bottom). The maximal decrease in HR (middle) was 61 bpm. (B) Analog wave forms detected in the neuron of panel A. (C) An intravenous bolus of CCK, which increased MAP (top) by 32 mm Hg, inhibited the spontaneous firing rate of this non-phasic-type neuron (100.0%, bottom). The maximal decrease in HR (middle) was 64 bpm. (D) Analog wave forms detected in the neuron of panel C.

pared to phasic-type neurons ($P<0.05$). There was no difference between the groups in terms of changes in MAP or HR.

4. Discussion

4.1. Spontaneous unit activity of the PVN under resting conditions

AVP-secreting neurons may be identified by their phasic firing patterns [8,38,39,59]. In the present study, we recorded such phasic neural activities within the PVN neurons in freely moving rats. Experiments using a dorsal approach in anesthetized rats have shown that about 20% of the antidromically identified PVN neurons displayed a phasic pattern [57,58]. Although we did not examine antidromic responses following stimulation of the posterior pituitary gland, about 25% of the neurons in the PVN region showed a phasic firing pattern. In particular, many phasic-type neurons were recognized in the PVN magnocellular area (Fig. 1B) [49]. In contrast, non-phasic-type neurons showed no discrete localization. These results are consistent with the characterization of AVP-secreting neurons as phasic-type neurons.

In phasic-type neurons, the mean duration of the active and silent phases was shorter under conscious conditions than under anesthetized conditions {mean active duration: 30.3 s (range 8.0–95.6 s), mean silent duration: 22.9 s (range 11.2–50.4 s)} [57]. In addition, the mean intraburst firing rates were lower under conscious conditions than under anesthetized conditions (3–15 spikes/s) [38]. There could be a direct effect of anesthesia on phase durations and firing rate.

4.2. PVN neuron response to various stressors

4.2.1. Baroreceptor activation and unloading

Baroreceptor activation induced by i.v. administration of PE [18,53] and other methods [24,38] inhibits hypothalamic neurosecretory neurons under anesthetized conditions. In contrast, AVP-secreting neurons may be activated by baroreceptor unloading [5,13,19]. In fact, neural activity in response to cardiovascular changes correlates with changes in plasma concentration of AVP. Thus, cardiovascular changes should be regarded as systemic stressors [42].

In the present study, a greater percentage of phasic-type neurons were inhibited by baroreceptor activation and excited by baroreceptor unloading (63.0%; 17/27, Table 2). In contrast, a greater percentage of non-phasic-type neurons showed no response to baroreceptor activation or baroreceptor unloading (70.3%; 52/74, Table 2). These results are closely related to data obtained under anesthesia [18,19,24,38,53].

The central neural pathways mediate inhibition of AVP in response to arterial baroreceptor activation through various routes. First, baroreceptor afferents excite a neuron in the nucleus tractus solitaris (NTS), leading to inhibition of the A1 noradrenaline (NA) neurons in the caudal ventrolateral medulla, probably via γ-aminobutyric acid (GABA) ergic neurons in the NTS. This leads to a withdrawal of the tonic excitatory input to the PVN and SON neurons [25]. Alternatively, the primary pathway for baroreceptor-derived inhibition of PVN and SON neurons may involve an excitatory A2 NA-utilizing pathway from the NTS to the diagonal band of Broca [27] and then a secondary excitatory projection to the GABAergic neurons with perikarya located dorsal to the PVN and SON [6]. Ultimately, the activity of these neurons is thought to inhibit AVP neurons.

4.2.2. Water deprivation

Dehydration and osmotic stimulation activate magnocellular neurosecretory cells [23,34,40,44]. Water deprivation increases plasma osmolality and reduces extracellular fluid, leading to intracellular dehydration [3] and thereby stimulating central osmoreceptors. These include the subfornical organ, organum vasculosum of the lamina terminalis, median preoptic nucleus, and connections with the magnocellular SON and PVN [1,17,21,32,34,36]. Consequently, water deprivation activates AVP secretion [12,23]. Thus, water deprivation can be considered a prototypical systemic, interoceptive stressor [42,52].

In the present study, dehydration primarily resulted in the activation of phasic-type neurons in the PVN and also in the activation of certain non-phasic-type neurons that may include oxytocin (OT)-secreting neurons (Table 1) [23]. Further, dehydration resulted in preferential increases in the intraburst firing rate over the interburst duration, which

Table 2
PVN neural response to altered hemodynamics

	Baroreceptor-activation							
	Phasic type				Non-phasic type			
	Excitation	Inhibition	No response	Total	Excitation	Inhibition	No response	Total
Baroreceptor-unloading								
Excitation	0	17	1	18	3	1	1	5
Inhibition	0	1	0	1	0	6	3	9
No response	1	6	1	8	4	4	52	60
Total	1	24	2	27	7	11	56	74

Phasic- (27 neurons) and non-phasic-type neurons (74 neurons) were classified on the basis of their responses to both baroreceptor activation and unloading.

contradicts previous reports of osmotic challenge under anesthetized conditions [21,36]. Water intake gradually attenuated the enhanced neural activities of phasic-type neurons (Fig. 5C). This finding contrasts with previous reports in monkeys, in which water intake abruptly inhibited neural activity of the neurosecretory neuron in the SON [56]. Although the reason for the discrepancy is not clear, it may be attributed to a species difference [38].

4.2.3. Air-jet stimulation

Air-jet stimulation has been regarded as a prototypical emotional, mild exteroceptive stressor [33,52]. The dorsomedial hypothalamic nucleus (DMN) had neural connections to the PVN that were influenced by air-jet stimulation-induced changes in cardiovascular and neuroendocrine function [33,47]. An anatomical tracing study [51] showed that the DMN projects heavily to the parvocellular region of the PVN, which contains CRH-secreting neurons that project to the ME. Thus, we speculated that air-jet stimulation-responsive neurons would contain certain parvocellular CRH-secreting neurons in the PVN through the DMN. In addition, air-jet stimulation increased the extracellular concentration of noradrenaline in the PVN region in conscious, freely moving rats [22].

In the present study, air-jet stimulation activated some non-phasic-type neurons with little response from phasic-type neurons. Onaka et al. [35] claimed that physical stress augments AVP secretion, while emotional stress results in the attenuation of AVP secretion. Thus, AVP secretion may be regulated differentially by intero- and exteroceptive stressors.

4.2.4. Systemic administration of CCK

CCK is one of the brain-gut peptides [4,45] that may participate in the neuroendocrine and autonomic responses to stressors [10]. Previous reports suggest that CCK-responsive neurons are OT-secreting neurons [53–55].

In the present study, CCK resulted in the activation of only excitatory non-phasic-type neurons. Conversely, all phasic-type neurons were inhibited by the administration of CCK. CCK plays multiple roles in homeostasis [45,54,55]; it is, for example, the prototypical putative satiety signal released in response to meal ingestion, and it decreases gastric emptying. Thus, although increased MAP could participate in the inhibition of phasic-type neurons, it would not be surprising if CCK had direct or other indirect effects on neuronal activity. Many studies have shown that systemic administration of CCK stimulates the pituitary secretion of OT, but not AVP, in rats [7,54], which is consistent with the present data.

4.2.5. Relationship between responses to air-jet stimulation and CCK administration

Interestingly, some authors have reported that CCK and CRH, both of which have been extensively studied in anxiety models [10,28], are co-localized within PVN neurons [31,41]. Generally, CCK is thought to induce anxiogenic-like effects, although studies have been highly variable and sometimes contradictory in this regard [10,28]. Table 3 shows the relationship between air-jet stimulation and i.v. administration of CCK on the PVN unit activity. Most non-phasic-type neurons that showed excitation in response to i.v. administration of CCK (88.9%; 8/9) did not respond to air-jet stimulation. Similarly, most non-phasic-type neurons that showed excitation in response to air-jet stimulation (90.0%; 9/10) did not show excitation in response to i.v. administration of CCK. Thus, stress induced by air-jet stimulation and by CCK appears to be processed in different subpopulations of non-phasic-type neurons in the PVN.

4.3. Functional implication of the PVN with single-unit recording in conscious animals

In the present study, we found that intero- and exteroceptive stressors differentially affect single neural activity of PVN neurons in conscious, freely moving rats. Stressors can be divided into two central stress circuit categories [14,22,42,52]. One is interoceptive (systemic) stress, which directly threatens survival and activates the PVN through the ascending catecholaminergic pathways from the brainstem [19,52], bypassing the need for cognitive processing. The other group consists of exteroceptive (processive) stressors, which are active in the cortical and limbic areas before the PVN is activated [14,42,52]. Signals from multiple sensory modalities are processed in these structures

Table 3
Relationship between responses to air-jet stimulation and CCK administration on PVN unit-activity

	CCK administration							
	Phasic type				Non-phasic type			
	Excitation	Inhibition	No response	Total	Excitation	Inhibition	No response	Total
Air-jet stimulation								
Excitation	0	0	0	0	1	7	2	10
Inhibition	0	0	0	0	0	4	0	4
No response	0	15	0	15	8	9	7	24
Total	0	15	0	15	9	20	9	38

Phasic- (15 neurons) and non-phasic-type neurons (38 neurons) were classified on the basis of their responses to both air-jet stimulation and CCK administration.

prior to final elaboration of the stress response. Lesions of the prefrontal cortex, hippocampus, and amygdala abolished autonomic and HPA responses to stress [11,16,20].

Much of the recent progress in identifying the individual hypothalamic effector neurons and extended circuitries that underlie adaptive responses to stress has come from the use of immediate-early genes (IEGs) as markers of neuronal activation [40,55,62]. Despite the usefulness of induced IEG expression to produce generic induction of synaptic stimulation, the local relationship of IEGs to the responsiveness of individual neurons remains uncertain. It is critical, therefore, to examine single-unit activity of PVN neurons under ''physiological'' conditions to elucidate the processing mechanisms for functional stress-related signals; in particular, responses to water deprivation followed by water intake and a mild exteroceptive stressor, such as air-jet stimulation, could be examined. This procedure provides feasibility data for further examination concerning stress responses.

In summary, our results provide limited support for the hypothesis that phasic-type neurons in the PVN are most likely AVP-secreting neurons in the conscious condition and that non-phasic-type neurons contain various types of neurons [15,29], which include putative OT- and CRH-secreting neurons in the PVN. Further studies are in progress utilizing an antidromic technique to identify the projections of the cells.

Acknowledgements

We appreciate Ms. Kumiko Iki's excellent technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research (14370024) from the Ministry of Education, Science, Sports, and Culture, Japan, and the Japanese COE Program (Section of Life Science). This study was also carried out as part of the ''Ground Research Announcement for Space Utilization'' promoted by the Japan Space Forum.

References

[1] J.W. Anderson, P.M. Smith, A.V. Ferguson, Subfornical organ neurons projecting to PVN: whole-cell properties, Brain Res. 921 (2001) 78–85.
[2] E. Arnauld, J.D. Vincent, J.J. Dreifuss, Firing patterns of hypothalamic supraoptic neurons during water deprivation in monkeys, Science 185 (1974) 535–537.
[3] C.C. Barney, R.M. Threatte, M.J. Fregly, Water deprivation-induced drinking in rats: role of angiotensin II, Am. J. Physiol. 244 (1983) R244–R248.
[4] M.C. Beinfeld, D.K. Meyer, M.J. Brownstein, Cholecystokinin octapeptide in the rat hypothalamo-neurohypophysial system, Nature 288 (1980) 376–378.
[5] G.W. Bisset, N.F. Fones, Antidiuretic Hormone. Recent Advances in Renal Disease, Churchill Livingstone, Edinburgh, 1975, pp. 350–416.
[6] C. Boudaba, K. Szabó, J.G. Tasker, Physiological mapping of local inhibitory inputs to the hypothalamic paraventricular nucleus, J. Neurosci. 16 (1996) 7151–7160.
[7] D.A. Carter, S.L. Lightman, A role for the area postrema in mediating cholecystokinin-stimulated oxytocin secretion, Brain Res. 435 (1987) 327–330.
[8] P. Cobbet, K.G. Smithson, G.I. Hatton, Immunoreactivity to vasopressin- but not oxytocin-associated neurophysin antiserum in phasic neurons of rat hypothalamic paraventricular nucleus, Brain Res. 362 (1986) 7–16.
[9] B.A. Cross, D. Novin, J.W. Sundsten, Antidromic activation of neurons in the paraventricular nucleus by stimulation in the neural lobe of the pituitary, J. Physiol. (Lond.) 203 (1969) 68P–70P.
[10] V. Daugé, I. Léna, CCK in anxiety and cognitive processes, Neurosci. Biobehav. Rev. 22 (1998) 815–825.
[11] D. Diorio, V. Viau, M.J. Meaney, The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic–pituitary–adrenal responses to stress, J. Neurosci. 13 (1993) 3839–3847.
[12] F.L. Dum, T.J. Brennan, A.E. Nelson, G.L. Robertson, The role of blood osmolality and volume in regulating vasopressin secretion in the rat, J. Clin. Invest. 52 (1973) 3212–3219.
[13] B. Egan, R. Grekin, H. Ibsen, K. Osterziel, S. Julius, Role of cardiopulmonary mechanoreceptors in ADH release in normal humans, Hypertension 6 (1984) 832–836.
[14] M.H. Emmert, J.P. Herman, Differential forebrain c-fos mRNA induction by ether inhalation and novelty: evidence for distinctive stress pathways, Brain Res. 845 (1999) 60–67.
[15] C. Fekete, G. Wittmann, Z. Liposits, R.M. Lechan, GABA-ergic innervation of thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus of the rat, Brain Res. 957 (2002) 251–258.
[16] S. Feldman, N. Conforti, A. Itzik, J. Weidenfeld, Differential effect of amygdaloid lesions on CRF-41, ACTH, and corticosterone responses following neural stimuli, Brain Res. 658 (1994) 21–26.
[17] A.V. Ferguson, T.A. Day, L.P. Renaud, Subfornical organ efferents influence the excitability of neurohypophyseal and tuberoinfundibular paraventricular nucleus neurons in the rat, Neuroendocrinology 39 (1984) 423–428.
[18] M.C. Harris, Effects of chemoreceptor and baroreceptor stimulation on the discharge of hypothalamic supraoptic neurons in rats, J. Endocrinol. 82 (1979) 115–125.
[19] M.C. Harris, K.M. Spyer, Neural regulation of vasopressin-containing hypothalamic neurons and the role of vasopressin in cardiovascular function, in: A.D. Loewy, K.M. Spyer (Eds.), Central Regulation of Autonomic Functions, Oxford Univ. Press, New York, 1990, pp. 224–246.
[20] J.P. Herman, M.K. Schäfer, E.A. Young, R. Thompson, J. Douglass, H. Akil, S.J. Watson, Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis, J. Neurosci. 9 (1989) 3072–3082.
[21] B.K. Honda, H. Negoro, R.E.J. Dyball, T. Higuchi, S. Takano, The osmoreceptor complex in the rat: evidence for interactions between the supraoptic and other deiencephalic nuclei, J. Physiol. 431 (1990) 225–241.
[22] Q.H. Jin, T. Kunitake, Y. Ishizuka, H. Kannan, Increase in norepinephrine but not nitric oxide metabolite levels in the hypothalamic paraventricular nucleus region in response to air-jet and swing rotation in freely moving conscious rats, Brain Res. 876 (2000) 205–210.
[23] M. Kadekaro, J.Y. Summy-long, S. Freeman, J.S. Harris, M.L. Terrell, H.M. Eisenberg, Cerebral metabolic responses and vasopressin and oxytocin secretions during progressive water deprivation in rats, Am. J. Physiol. 262 (1992) R310–R317.
[24] H. Kannan, H. Yamashita, Electrophysiological study of paraventricular nucleus neurons projecting to the dorsomedial medulla and their response to baroreceptor stimulation in rats, Brain Res. 279 (1983) 31–40.
[25] H. Kannan, T. Osaka, M. Kasai, S. Okuya, H. Yamashita, Electro-

physiological properties of neurons in the caudal ventrolateral medulla projecting to the paraventricular nucleus of the hypothalamus in rats, Brain Res. 376 (1986) 342–350.
[26] H. Kannan, Y. Hayashida, H. Yamashita, Increase in sympathetic outflow by paraventricular nucleus stimulation in awake rats, Am. J. Physiol. 256 (1989) R1325–R1330.
[27] M. Kasai, T. Osaka, K. Inenaga, H. Kannan, H. Yamashita, γ-Aminobutyric acid antagonist blocks baroreceptor-activated inhibition of neurosecretory cells in the hypothalamic paraventricular nucleus of rats, Neurosci. Lett. 81 (1987) 319–324.
[28] S. Kõks, P.T. Männistö, M. Bourin, J. Shlik, V. Vasar, E. Vasar, Cholecystokinin-induced anxiety in rats: relevance of pre-experimental stress and seasonal variations, J. Psychiatry Neurosci. 25 (2000) 33–42.
[29] D. Lawrence, Q.J. Pittman, Response of rat paraventricular neurones with central projections to suckling, haemorrhage, or osmotic stimuli, Brain Res. 341 (1985) 176–183.
[30] I. Merchenthaler, M.A. Hynes, S. Vigh, A.V. Schally, P. Petrusz, Corticotropin-releasing factor (CRF): origin and course of afferent pathways to the median eminence (ME) of the rat hypothalamus, Neuroendocrinology 39 (1984) 296–306.
[31] E. Mezey, T.D. Reisine, L. Skirboll, M. Beinfeld, J.Z. Kiss, Cholecystokinin in the medial parvocellular subdivision of the paraventricular nucleus. Co-existence with corticotropin-releasing hormone, Ann. N. Y. Acad. Sci. 448 (1985) 152–156.
[32] R.R. Miselis, The efferent projections of the subfornical organ of the rat: a circumventricular organ within a neural network subserving water balance, Brain Res. 230 (1981) 1–23.
[33] S.M. Morin, E.H. Stotz-Potter, J.A. DiMicco, Injection of muscimol in dorsomedial hypothalamus and stress-induced Fos expression in paraventricular nucleus, Am. J. Physiol. 280 (2001) R1276–R1284.
[34] B.J. Oldfield, R.J. Bicknell, R.M. McAllen, R.S. Weisinger, M.J. McKinley, Intravenous hypertonic saline induces Fos immunoreactivity in neurons throughout the lamina terminals, Brain Res. 561 (1991) 151–156.
[35] T. Onaka, M. Hamamura, K. Yagi, Suppression of vasopressin secretion by classically conditioned stimuli in rats, Jpn. J. Physiol. 36 (1986) 1261–1266.
[36] T. Osaka, H. Kannan, M. Kasai, K. Inenaga, H. Yamashita, Osmotic response of rat paraventricular neurons by pressure ejection method, Brain Res. Bull. 24 (1990) 493–497.
[37] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, 2nd ed., Academic Press, New York, 1986.
[38] D.A. Poulain, J.B. Wakerley, Electrophysiology of hypothalamic magnocellular neurons secreting oxytocin and vasopressin, Neurosci. 7 (1982) 773–808.
[39] D.A. Poulain, J.B. Wakerley, R.E.J. Dyball, Electrophysiological differentiation of oxytocin- and vasopressin-secreting neurons, Proc. R. Soc. B. 196 (1977) 367–384.
[40] S.M. Sagar, F.R. Sharp, T. Curran, Expression of c-fos protein in brain: metabolic mapping at the cellular level, Science 240 (1988) 1328–1331.
[41] P.E. Sawchenko, L.W. Swanson, W.W. Vale, Corticotropin-releasing factor: co-expression within distinct subsets of oxytocin-, vasopressin-, and neurotensin-immunoreactive neurons in the hypothalamus of the male rat, J. Neurosci. 4 (1984) 1118–1129.
[42] P.E. Sawchenko, H.Y. Li, A. Ericsson, Circuits and mechanisms governing hypothalamic responses to stress: a tale of two paradigms, Prog. Brain Res. 122 (2000) 61–78.
[43] A. Shimokawa, Q.H. Jin, Y. Ishizuka, T. Kunitake, M. Takasaki, H. Kannan, Effects of anesthetics on norepinephrine release in the hypothalamic paraventricular nucleus region of awake rats, Neurosci. Lett. 244 (1998) 21–24.
[44] C.D. Sladek, J.A. Olschowka, Dehydration induces Fos, but not increased vasopressin mRNA in the supraoptic nucleus of aged rats, Brain Res. 652 (1994) 207–215.
[45] G.P. Smith, C. Jerome, B.J. Cushin, R. Eterno, K.J. Simansky, Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat, Science 213 (1981) 1036–1037.
[46] R.L. Stornetta, P.G. Guyenet, R.C. McCarty, Autonomic nervous system control of heart rate during baroreceptor activation in conscious and anesthetized rats, J. Auton. Nerv. Syst. 20 (1987) 121–127.
[47] E.H. Stotz-Potter, S.M. Morin, J.A. DiMicco, Effect of microinjection of muscimol into the dorsomedial or paraventricular hypothalamic nucleus on air stress-induced neuroendocrine and cardiovascular changes in rats, Brain Res. 742 (1996) 219–224.
[48] A.J.S. Summerlee, A.C. Praisley, K.T. O'Byrne, K.M. Fairhall, I.C.A.F. Robinson, J. Fletcher, Aspects of the neuronal and endocrine components of reflex milk ejection in conscious rabbit, J. Endocrinol. 108 (1984) 143–149.
[49] L.W. Swanson, P.E. Sawchenko, Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms, Neuroendocrinology 31 (1980) 410–417.
[50] L.W. Swanson, P.E. Sawchenko, R.W. Rind, Regulation of multiple peptides in CRF parvocellular neurosecretory neurons: implications for the stress response, Prog. Brain Res. 68 (1986) 169–190.
[51] G.J. Ter Horst, P.G. Luiten, *Phaseolus vulgaris* leuco-agglutinin tracing of intrahypothalamic connections of the lateral, ventromedial, dorsomedial, and paraventricular hypothalamic nuclei in the rat, Brain Res. Bull. 18 (1987) 191–203.
[52] K.V. Thrivikraman, C.B. Nemeroff, P.M. Plotsky, Sensitivity to glucocorticoid-mediated fast-feedback regulation of the hypothalamic–pituitary–adrenal axis is dependent upon stressor-specific neurocircuitry, Brain Res. 870 (2000) 87–101.
[53] Y. Ueta, H. Kannan, T. Higuchi, H. Negoro, H. Yamashita, CCK-8 excites oxytocin-secreting neurons in the paraventricular nucleus in rats—possible involvement of noradrenergic pathway, Brain Res. Bull. 32 (1993) 453–459.
[54] J.G. Verbalis, M.J. McCann, C.M. McHale, E.M. Stricker, Oxytocin secretion in response to cholecystokinin and food: differential of nausea from satiety, Science 232 (1986) 1417–1419.
[55] J.G. Verbalis, E.M. Stricker, A.G. Robinson, G.E. Hoffman, Cholecystokinin activates c-fos expression in hypothalamic oxytocin and corticotropin-releasing hormone neurons, J. Neuroendocrinol. 3 (1991) 205–213.
[56] J.D. Vincent, E. Arnauld, B. Bioulac, Activity of osmosensitive single cells in the hypothalamus of the behaving monkey during drinking, Brain Res. 44 (1972) 371–384.
[57] J.B. Wakerley, D.W. Lincoln, Phasic discharge of antidromically identified units in the paraventricular nucleus of the hypothalamus, Brain Res. 25 (1971) 192–194.
[58] J.B. Wakerley, D.W. Lincoln, The milk-ejection reflex of the rat: a 20- to 40-fold acceleration in the firing of paraventricular neurones during oxytocin release, J. Endocrinol. 57 (1973) 477–493.
[59] J.B. Wakerley, D.A. Poulain, R.E.J. Dyball, B.A. Cross, Activity of phasic neurosecretory cells during hemorrhage, Nature 258 (1975) 82–84.
[60] Z. Yang, J.H. Coote, The influence of the paraventricular nucleus on baroreceptor-dependent caudal ventrolateral medullary neurons of the rat, Pflugers Arch. 438 (1999) 47–52.
[61] X. Zhang, R. Fogel, W.E. Renehan, Stimulation of the paraventricular nucleus modulates the activity of gut-sensitive neurons in the vagal complex, Am. J. Physiol. 277 (1999) G79–G90.
[62] W. Zhu, H. Umegaki, J. Yoshimura, N. Tamaya, Y. Suzuki, H. Miura, A. Iguchi, The elevation of plasma adrenocorticotrophic hormone and expression of c-Fos in hypothalamic paraventricular nucleus by microinjection of neostigmine into the hippocampus in rats: comparison with acute stress responses, Brain Res. 892 (2001) 391–395.
[63] M. Zimpfer, W.T. Manders, A.C. Barger, S.F. Vatner, Pentobarbital alters compensatory neural and humoral mechanisms in response to hemorrhage, Am. J. Physiol. 243 (1982) H713–H721.