“On-” and “off-” cells in the rostral ventromedial medulla of rats held in thermoneutral conditions: are they involved in thermoregulation?

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Submitted 7 October 2013; accepted in final form 8 July 2014

El Bitar N, Pollin B, Le Bars D. “On-” and “off-” cells in the rostral ventromedial medulla of rats held in thermoneutral conditions: are they involved in thermoregulation? J Neurophysiol 112: 2199–2217, 2014. First published July 9, 2014; doi:10.1152/jn.00722.2013.—In thermal neutral condition, rats display cyclic variations of the vasomotion of the tail and paws, synchronized with fluctuations of blood pressure, heart rate, and core body temperature. “On-” and “off-” cells located in the rostral ventromedial medulla, a cerebral structure implicated in somatic sympathetic drive, 1) exhibit similar spontaneous cyclic activities in antiphase and 2) are activated and inhibited by thermal nociceptive stimuli, respectively. We aimed at evaluating the implication of such neurons in autonomic regulation by establishing correlations between their firing and blood pressure, heart rate, and skin and core body temperature variations. When, during a cycle, a relative high core body temperature was reached, the on-cells were activated and within half a minute, the off-cells and blood pressure were depressed, followed by heart rate depression within a further minute; vasodilatation of the tail followed invariably within ~3 min, often completed with vasodilatation of hind paws. The outcome was an increased heat loss that lessened the core body temperature. When the decrease of core body temperature achieved a few tenths of degrees, sympathetic activation switches off and converse variations occurred, providing cycles of three to seven periods/h. On- and off-cell activities were correlated with inhibition and activation of the sympathetic system, respectively. The temporal sequence of events was as follows: core body temperature → on-cell → off-cell → blood pressure → heart rate → skin temperature → core body temperature. The function of on- and off-cells in nociception should be reexamined, taking into account their correlation with autonomic regulations.

thermoregulation; pain modulation; blood pressure variability; rostral ventromedial medulla; rostral medullary raphe

WHETHER IN ACUTE OR CHRONIC models, the tail and the paws are the most used target organs to study pain in rodents. It is an implicit assumption that such experiments must be performed on organisms maintained in stable physiological equilibrium. In endothermic species, the preservation of the core body temperature in a narrow range is one of the very first principles, which govern the stability of the “milieu intérieur” required for a free and independent life (Bernard 1865; Cannon 1932). The core body temperature results from the balance between heat production and heat loss (Gordon 1990, 1993; Romanovsky et al. 2002): thermal receptors located in the skin and body core send information to specific relays within the brain to adjust thermoregulatory processes such that an increased warm signal input activates heat loss, while an increased cold signal input increases insulation and heat production. However, the state of thermal neutrality is not characterized by an atony of the thermoregulatory processes. We recently described in the rat that thermal neutrality is achieved by large cyclic variations of the temperature of the tail and hind paws that are secondary to vasomotor tone variations, themselves associated with fluctuations of the blood pressure and heart rate (HR; El Bitar et al. 2014; Young and Dawson 1987).

The frequency domain of these variations peaked at 0.001–0.002 Hz for all signals, that is a few cycles per hour. Such domain fit the so-called “extremely low frequencies” recognized during long-lasting recordings of blood pressure or HR variability (Julien et al. 2008; Persson et al. 1992), themselves fitting the patterns of spontaneous variations of the firing of neurons recorded in the rostral ventromedial medulla (RVM; Leung and Mason 1996; Thurston and Randich 1992, 1995). Following the proposition by Mason (2001, 2005a, 2005b, 2006, 2011), we decided to assemble in a single entity two largely overlapping brain regions, the RVM, as defined in the pain-related literature (Fields et al. 2006), and the rostral medullary raphe (rMR), as defined in the thermoregulation-related literature (Blessing 2003; McAllen et al. 2010; Morrison 2011; Morrison and Nakamura 2011; Nakamura and Morrison 2008). Thus the “RVM/rMR” includes the raphe pallidus, raphe magnus, parapyramidal nucleus, and the reticular formation that extends under the gianocellular reticular nucleus.

RVM/rMR neurons send axons through the dorsolateral funiculus (DLF) in the spinal cord not only towards the dorsal horn (Fields and Basbaum 1978, 1999) but also towards the intermediolateral cell column (Bacon et al. 1990; Basbaum and Fields 1979; Hossaini et al. 2012; Lefler et al. 2008; Loewy 1981; Morrison and Gebber 1985). Since 1) most experiments examining a role for RVM/rMR in nociception were made under anesthetic regimes that abolished withdrawal reflexes and have not recorded thermoregulatory (nor cardiovascular in most cases) variables and 2) most experiments examining a role for RVM/rMR in thermoregulation were made under anesthetic regimes that abolish withdrawal reflexes, the question of the interaction between these functions remained largely open at this level. We aimed here at evaluating the implication of the “on-” and “off-” cells, as defined by Fields and coworkers (Fields et al. 1983, 1988; Vanegas et al. 1984), in autonomic regulation by establishing correlations between their firing and blood pressure, HR, skin, and core body temperature variations.
**MATERIALS AND METHODS**

**Ethical Statement**

Animal experiments were performed with permission of the Board of the Veterinarian Services of the French Ministry of Agriculture (permit number 75–151) in accordance with the National Institute of Health’s Guide for the Care and Use of Laboratory Animals, the European Communities Council Directive 86/609/EEC regulating animal research, and the Ethics Committee of the International Association for the Study of Pain (Covino et al. 1980; Zimmermann 1983). The procedures were approved by the Committee of Ethics for the Animal Experimentation of our Institution.

**Animals**

A total of 32 adult male Sprague-Dawley rats (Janvier Labs, Saint-Berthevin, France) took part in the experiments, weighing 320–370 g. They were housed in groups of three to four per cage, allowed free access to food and water with a 12 h alternating light-dark cycle, and acclimatized to the laboratory for at least one wk before the experiment. The experiments were conducted between 9 AM and 5 PM.

**Experimental Procedure**

The animals were deeply anesthetized with 2.5% halothane in 100% oxygen. A tracheal cannula was inserted following subcutaneous injection of 0.5 ml xylocaine 2%, and the ventilation was controlled mechanically with an open circuit respirator equipped with a scavenging system, at a rate of 50 counts/min. The expiratory halothane level and end-tidal CO₂ were assessed with a capnometer (Capnomac II; Datex Instruments, Helsinki, Finland), sampled each 10 s and under control of alarms throughout the experiment. A catheter was inserted into the common carotid artery to monitor arterial blood pressure and HR. The rat was mounted on a Horsley-Clarke stereotaxic frame. 0.5 ml of xylocaine 2% was injected subcutaneously in the scalp, followed by a midline incision. After trepanation, a small incision of the dura matter was made to introduce a recording electrode. The electrodes were glass micropipettes with filaments filled with a solution of KC1 (0.75 M) colored with Pontamine sky blue. The glass micropipettes were made from glass capillary tubes fixed by the chuck of the drill of a Narashige vertical puller model PE-2. It was stretched by using both the gravitational force of its own weight and the force of the magnet. The diameter of the tip of the electrode was ~1 μm. Their impedance was 8–12 MΩ.

The pipette was connected to an amplifier then to LABCHARt data acquisition software (ADInstruments). The tip of the electrode was inserted to the RVM/rMR. After surgery, halothane was optimized adjusted slightly above the average of core body temperature to maintain the rats in thermoneutral conditions, as previously described (El Bitar et al. 2014), 0.4°C higher than the core body temperature estimated around half a minute (El Bitar et al. 2014). Such an lag between vasodilatation and skin temperature increase was recently verified (El Bitar et al. 2014). The mean time blood flow and the thermographic measure of surface skin temperature was adjusted to keep the end tidal CO₂ ~3.5%. Experimental measures started at least 30 min after the end of surgery.

To maintain the anesthetized rat in a thermoneutral condition, the body of the animal was wrapped up in a water filled warming pad connected to an extra-capacity water circulator (TP 220; Kent Scientific) sparing the head, the paws, and the tail. The heating blanket was covered with an isothermic metalized polyester film to stabilize the space temperature around the body. The warming temperature was adjusted slightly above the average of core body temperature to maintain the rats in thermoneutral conditions, as previously described (El Bitar et al. 2014). Under stable environmental temperature, skin temperature is a reliable indicator of skin blood flow variations (Hertzman 1953). The strong correlation between the laser-Doppler measure of skin blood flow and the thermographic measure of surface skin temperature was verified, recently (El Bitar et al. 2014). The mean time lag between vasodilatation and skin temperature increase was estimated around half a minute (El Bitar et al. 2014). Such an approach has the advantage of giving accurate measures of the temperatures at both temporal and spatial levels in the form of a thermal imaging.
An infrared camera (JADE MWIR; CEDIP Infrared Systems, Croissy-Beaubourg, France) with a 3- to 5-μm optical bandpass and a 500-μs integration time was used to obtain images of 320 × 240 pixels at a 1-Hz sampling rate, with a sensitivity of 0.02°C at 25°C. The camera was placed 1.5 m above the scene and was controlled by the software Cirrus (CEDIP Infrared Systems). The camera was calibrated using a black body as previously described (Benoist et al. 2008). The software Altair (CEDIP Infrared Systems) was used to explore the spatial and temporal dynamics of the skin temperature at the level of the tail and paws, as follows.

First, several regions of interest (ROI) were defined in the recorded scene, each comprising 10 pixels. Three ROIs were located on the tail. A proximal ROI (tail-prox) was placed 3 cm from the root of the tail, an intermediate ROI (tail-mid) was placed at the middle of the tail, and a distal ROI (tail-distal) was placed 3 cm from the tip of the tail. Two additional ROIs were located on the plantar aspect of the left and right hind paws of the anesthetized rats (paw-left and paw-right). Finally, a ROI was located over a piece of wood placed in the scene to monitor the ambient temperature (ambient). For each time point, the mean of the 10 pixels defining each ROI was computed to obtain one single temperature time course for each ROI (Ttail-prox, Ttail-mid, Ttail-distal, Tpaw-left, Tpaw-right, and Tambient). To simplify the presentation, Ttail-mid and Tpaw-right will be considered as representative for the tail and hind paws, respectively (El Bitar et al. 2014).

**Cells Selection for Recording**

Cells candidates for analysis were located in the RVM/rMR mainly at −1.7 to −2.5 mm caudal to the interaural line (Fig. 1). On- and off-cells were identified on the basis of the criteria described by Fields et al. (1983, 1988) during a tail-flick response to radiant heat: on- and off-cells exhibit a sudden increase and an abrupt pause in firing rate, respectively, before the occurrence of the tail-flick. The tail-flick tests were performed using an Ugo Basile apparatus (7370) with the stimulus being placed halfway between intermediate and distal ROIs, as defined above. The infrared Source is lodged into a metal frame containing a halogen bulb (Halogen “Bellaphot,” model 64607 OS-RAM, 8V-50W) coupled to an infrared filter that cuts off most visible part of the spectrum, cooled by an operating fan. The Controller 7371 records the stimulation time from the moment the start key is activated to the moment the animal withdraws the tail with a 0.1-s accuracy (see details on: http://ugobasile.com/support/documentation/viewcategory/3.html). It was connected through an amplifier to LABCHART data acquisition software (AD Instruments) together with the electrophysiological signal. The operator started the stimulus, and a sensor detected the tail withdrawal, stopped the timer and switched off the bulb. We used a 10-s cutoff time.

**Histological Controls**

At the conclusion of the experiment, the rat was deeply anesthetized with 3% halothane and the brain was perfused through the heart with 0.9% NaCl, followed by 10% formaldehyde and removed. The brain was frozen, cut in serial 100-μm thick sections and Nissl-stained with cresyl violet or carmin. Sites of microinjections were determined from microscopic visualization of the serial sections and reported on schemas of frontal sections of the brain (Paxinos and Watson 2005), as shown in Fig. 1.

**Arterial Blood Pressure and HR**

The arterial blood pressure and HR were monitored continuously using the catheter inserted into the common carotid artery, using a transducer connected to a computer. The mean arterial blood pressure (MAP) and HR were calculated using LABCHART data acquisition software (ADInstruments). Data acquisition was performed at a rate of 4 KHz and resampled according to the need.

**Core and Heating Temperature**

A two-channel OMEGA HH506RA digital thermometer and two VIP-T-CT25515 Probes (0.1°C resolution) were used to measure the core body temperature (Tcore). The probe was inserted 6 cm in the rectum (Lomax 1966). The second probe was placed around the trunk of the rat, such as to estimate the heating provided by the isothermal warming blanket. Each measurement was sampled every 60 s.

**Data Processing and Statistical Analysis**

Seventeen off-cell and 15 on-cell stable sequences lasting between 60 min and 2 h and 30 min were considered. All signals were resampled and synchronized at 1-Hz resolution. They were analyzed using Matlab R2006A (The MathWorks) as follows:

1) Descriptive statistics. Data were represented as mean with their 95% confidence interval.
2) Spectral analysis. A fast Fourier transform was used to estimate the frequency distribution of fluctuations during thermoneutral conditions.
3) Correlation analysis. Cross-correlations and Lissajous plots were used to study the linear correlation and lag between variables. The relative delay at which maximum correlation was observed was used to estimate the lag between two variables. Lissajous curves were plotted using Excel (Microsoft) to provide a convenient summary of the linear correlation, temporal lagging, and amplitude of fluctuations between two cyclic variables.
4) Synchronization. Since the MAP drops appeared the most stereotyped cardiovascular changes following on-cell activation or off-cell depression, their origins of time were chosen to synchronize the recordings to compare the results related to on- and off-cells on a chronological ground. The occurrence of such events was detected by marking out the maximal negative slopes of the derivative dMAP/dt, witness of the fastest MAP drops. Individual episodes were collected that began 3 min before and ended 6 min following such a realigned origin of time with the first 2-min period being taken as reference.
5) Statistical comparisons were done using the Mann-Whitney U test. Significance level was set at _P_ < 0.05.
RESULTS

Characterization of Recorded On- and Off-Cells

With the use of a conventional radiant heat device to stimulate the skin, the firing of the on-cells increased (Fig. 2A) while the firing of the off-cells decreased (Fig. 2B) before the occurrence of the tail-flick, as already described (Fields et al. 1983, 1988). By adjusting the origin of the time scale of each individual recording to the actual time of the tail-flick, the overall mean timing of events is illustrated in Fig. 2C for the

![Fig. 2. Identification of on- and off-cells. A: individual example of the response of an on-cell (red color) to radiant heat (scales in the right inset). Time zero represents the tail-flick occurrence, and 4.3 s is the reaction time or tail-flick “latency” (TFL; yellow horizontal bar). Note that the neuronal burst of activity started before the behavioral response at time zero. B: individual example of the response of an off-cell (blue color) to radiant heat. Note that the cessation of the neuronal activity started before the behavioral response at time zero (TFL = 3.1 s, yellow horizontal bar). C: mean firing of neurons recorded during tail-flick tests. Abscissa: time in seconds with origin of time being adjusted to the occurrence of the behavioral response. Ordinate: spikes/s (bw, bin width: 0.1 s) ± 95% confidence interval. In red is represented the mean of 31 tail-flick assays performed during the recording of 11 on-cells. Note that the activation of on-cell was observed before the response, but the peak of activity was seen 0.4 s following the tail-flick. In blue are represented the mean of 23 tail-flick assays performed during the recording of 9 off-cells. Note that the depression of off-cell was observed before the response, but the nadir was 0.5 s after the tail-flick. The very moment when the firing reached 50% of the total change is indicated by a white dot (1.1 and 0.4 s before the tail-flick, for on- and off-cells, respectively). Interestingly, these changes outlasted by 0.4–0.5 s the ending of the stimulation by the movement. There was no statistical difference between the mean tail-flick “latencies” measured during the recordings of on- and off-cells. The yellow haziness represents the stimulation time [mean TFL = 4.6 (4.3–4.9) s]. D: corresponding variations of mean arterial blood pressure (MAP) and heart rate (HR).]
on- and off-cells, respectively. Figure 2D summarizes the variations of MAP and HR. Note that a drop of MAP peaking within 2–3 s followed the withdrawal responses. In all cases the discharge changes for cells occurred ~2.5 s before the motor response and outlasted by 0.4–0.5 s the ending of the stimulation by the movement.

A total of 17 on-cells and 15 off-cells were recorded for ≥60 min from rats maintained in thermoneutral conditions. Overall, their mean firings were 5 (2–8) and 10 (7–12) spikes/s, respectively. These activities were characterized by large cyclic variations, as described below. The mean minimal and maximal firing rates of on-cells were 0 (0–0) and 29 (20–38) spikes/s, respectively; for off-cells, they were 1 (0–2) and 27 (22–32) spikes/s, respectively. The recording sites were all located within the RVM/rMR, 1.7–2.5 mm caudal to the interaural line, in the coronal planes that include the facial nuclei (Fig. 1).

A Typical Example of On-Cell Recording in a Rat Maintained in Thermoneutral Condition

Figure 3 provides a typical example of a 156-min recording of an on-cell localized in the raphe magnus (Fig. 3H; see also Supplemental Video S1; Supplemental Material for this article is available online at the J Neurophysiol website). Figure 3A shows the neuronal firing characterized by periodical bursts of activity, interrupted by silent periods. Similar periodic fluctuations were seen regarding MAP and HR (Fig. 3B). Figure 3C shows the temperature time courses of I) the environment (Tambient); 2) the skin measured at three sites of the tail (Ttail-prox, Ttail-mid, and Ttail-dist); and 3) the skin measured at the left and right paws (Tpaw-left and Tpaw-right). Skin temperatures also exhibited similar spontaneous, periodical fluctuations. The wavering corresponded to vasomotor tone variations, the vasocstriction and vasodilatation decreasing and increasing of skin temperature Tskin, respectively (El Bitar et al. 2014). The core body temperatures (Tcore; Fig. 3D) presented tiny fluctuations, opposite to the variations of Tskin. The spectral analysis of the variations revealed a major peak at 0.0008 Hz for all signals (Fig. 3G).

The dark grey columns in Fig. 3A were adjusted to the period of on-cell activation and extended to cover Fig. 3, B–D. They fitted the periods of MAP and HR decline (Fig. 3B) and slightly preceded the increases of Tskin (Fig. 3C). The temporal relation between the cellular activity and Tcore is emphasized in Fig. 3D: the firing started during the highest values (red points) of a Tcore cycle and ceased at the end of the following descending period (blue points), providing a complete cycling. These mean temporary set points were 0.07 (0.04–0.1)°C above and below the mean Tcore (38.0°C).

Averaged signals of the on-cell firing are provided in Fig. 3Ea, with the origin of time being realigned to the very beginning of six successive complete periods of on-cell activation. The corresponding temporal evolution of the other variables is shown in Fig. 3Eb in terms of z-scores with reference to the 3-min prefiring period. The chronology of events was confirmed, with a small delay for the MAP/HR drop and the 2–4 min delays (see horizontal arrows) for the vasodilatation of the skin. The corresponding temporal evolution of mean Tcore (Fig. 3Ec) clearly shows that the mean activating period started and ceased when Tcore achieved amazing precise values, namely 38.08 (38.04–38.11) in blue and 37.92 (37.86–37.98)°C in red, respectively. The decrease of Tcore began ~4 min following the burst start (black horizontal arrow). The corresponding relationship between the variations of the firing rate of the on-cell and the variations of MAP is provided in Fig. 3F: roughly, MAP decreased by 10 mmHg when the firing increased by one spike/s.

Figure 4 highlights the temporal relationship between the fluctuations of the firing of the cell and the other variables, shown in terms of z-scores. The Lissajous curves describe the correlation and time lag between variables (Fig. 4A). The strong correlation (absolute maximum Pearson’s correlation >0.6) between the measured variables and the firing rate of the on-cell was confirmed with cross-correlation analysis. Within each recording, the time lag was estimated by finding the delay at which absolute maximum correlation was found between the two signals. These time lags were then taken into account to build the lag-realigned Lissajous curves in Fig. 4B, which improved the correlations of the variables with the firing rate of the on-cell (yellow background insets). The regressions lines (solid black lines) show the phase shift between events, with roughly two cases: inverted (Fig. 4, Ba and Bd) or in phase (Fig. 4, Bb and Bc).

In summary, the on-cell firing, MAP, HR, Tskin, and Tcore were highly correlated. Firing changes were concomitant with MAP and preceded HR and Tcore variations in opposition of phase and Tskin changes in phase. The chronology of changes was as follows: on-cell → blood pressure → heart rate → skin temperature → core body temperature.

A Typical Example of Off-Cell Recording in a Rat Maintained in Thermoneutral Condition

Figure 5 provides a typical example of an 80-min recording of an off-cell localized in the raphe pallidus (Fig. 5H; see also Supplemental Video S2). Figure 5A shows that the neuronal firing oscillated in the ~0–20 spikes/s range at a ~5/6 cycles/h frequency. Similar periodic fluctuations were seen regarding MAP and HR (Fig. 5B). The corresponding variations of the temperature on the skin regions of interest are shown in Fig. 5C. The temperatures of the tail exhibited spontaneous, regular fluctuations; the hind paws were not always involved, but when they were, this was in synchrony with the tail pattern. Tcore (Fig. 5D) presented small fluctuations, opposite to the variations of Tskin. The spectral analysis of the variations revealed a major peak at 0.0016 Hz for all signals and some additional for the skin temperatures (Fig. 5G).

The dark grey columns adjusted to the periods of off-cell activation (Fig. 5A) were extended to cover Fig. 5, B–D. They fitted the period of elevation of MAP and HR (Fig. 5B) and preceded slightly the skin temperature decline (Fig. 5C). Quasi-constant marks were made on Tcore time course in Fig. 5D, at the onset and ending of the activation periods. These marks form the set points of Tcore representing thresholds for activating (red dots) and inhibiting (blue dots) the system for thermoregulatory purposes, and delimiting a thin interthresholds range.

Averaged signals of off-cell firing are provided in Fig. 5Ea, with the origin of time being realigned to the very beginning of seven successive periods of off-cell activation. The corresponding time evolution of the other variables is shown in Fig.
$5Eb$ in terms of $z$-scores with reference to the 3-min prefriring period. The chronology of events was confirmed: 1) an instantaneous MAP/HR rise, and 2) 2- to 4-min delays for the peripheral vasoconstrictions (horizontal arrows). The corresponding temporal evolution of $T_{\text{core}}$ shows that the mean activating period started and broke off when $T_{\text{core}}$ achieved slightly high and low values, respectively (Fig. 5Ec). It is a fact that the increase of $T_{\text{core}}$ began ~6–7 min following the burst start (black horizontal arrow). The corresponding relationship between the mean firing rate of the off-cell and the mean contemporaneous MAP is provided in Fig. 5F: beyond three spikes/s, an increased firing by five spikes/s was associated with a 10-mmHg MAP increase.

Figure 6 highlights the temporal relationship between the fluctuations the firing of the cell, and the other variables are shown in terms of $z$-scores. The Lissajous curves describe the corresponding evolution of the core body temperature expressed in terms of mean variation with reference to the 3-min prefriring period ($\Delta T_{\text{core}}$).
roughly two cases: inverted (Fig. 6, black lines) show the phase shift between events, with changes in opposition of phase. The chronology of changes in MAP and HR and preceded Tskin and Tcore variations in phase and Tskin.

Global Data

A series of on-cells in rats maintained in thermoneutral condition. The experiment described above was replicated with 17 on-cells in 17 rats maintained in thermoneutral condition. Cyclic synchrony and correlations of signals as observed in the example shown in Fig. 3 were reproduced. A spectral analysis of the signals showed that fluctuations occurred in the 0.0003- to 0.006-Hz range with a peak at 0.002 Hz for all signals (Fig. 7A). Note an eminent additional peak frequency at 0.17 Hz observed for MAP and HR together with others in the 0.2–0.5 Hz range, referred as low frequencies (Julien et al. 2008; Leung and Mason 1996; Persson et al. 1992; Stauss 2007). Cross-correlation analyses were also performed between each couple of variables, filtered to the determined frequency domain. Results of absolute maximal correlations and the corresponding time lags are presented in Table 1. The chronology of events shown in the individual experiment of Fig. 3 was rigorously confirmed: the beginning of on-cell firing was I) concomitant with the achievement of the highest values of
T<sub>core</sub> seen during the stationary recordings [in relative terms, 0.09 (0.03–0.16)°C above the mean T<sub>core</sub>], 2) quasi-instantaneous with the drop in MAP, followed by 3) a HR drop within 1 min, 4) a covariant variations of skin temperature within 2–4 min, and 5) a reverse variations of T<sub>core</sub> within 4–8 min. The opposite events happened when a T<sub>core</sub> set point of 0.06 (0.02–0.10)°C lower than the mean T<sub>core</sub> was achieved.

A series of off-cells in rats maintained in thermoneutral condition. The experiment described above was replicated with 15 off-cells in 15 rats maintained in thermoneutral condition. Cyclic synchrony and correlations of signals as observed in Fig. 6 were reproduced. A spectral analysis of the signals showed that fluctuations occurred in the 0.0003–0.006 Hz range with a peak between 0.001 and 0.002 Hz for all signals.
The experiments described above were performed on the basis of single unit recordings of either on- or off-cells, and no conclusion can be drawn directly regarding the chronologic order of on- and off-cell activities. It was obvious that the most time-locked and short-duration events were the sudden drops of changes was as follows: on-cells firing → off-cells firing → blood pressure → heart rate → skin temperature → core body temperature.

In thermoneural conditions, the rise or diminution of either on- or off-cell activities appeared strongly related to relative Tcore set points. When the core body temperature increased by very few tenths of degrees above the mean Tcore, on-cells were activated and off-cells inhibited. The opposite phenomenon occurred when Tcore decreased by very few tenths of degrees below the mean Tcore. Comparison of on- and off-cells relative set points did not reveal statistical differences ($P = 0.30$ and $0.24$, respectively). If one considers the other variables recorded in rats maintained in thermoneural condition, the two groups of experiments were comparable.

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The experiments described above were performed on the basis of single unit recordings of either on- or off-cells, and no conclusion can be drawn directly regarding the chronologic order of on- and off-cell activities. It was obvious that the most time-locked and short-duration events were the sudden drops of changes was as follows: on-cells firing → off-cells firing → blood pressure → heart rate → skin temperature → core body temperature.

In thermoneural conditions, the rise or diminution of either on- or off-cell activities appeared strongly related to relative Tcore set points. When the core body temperature increased by very few tenths of degrees above the mean Tcore, on-cells were activated and off-cells inhibited. The opposite phenomenon occurred when Tcore decreased by very few tenths of degrees below the mean Tcore. Comparison of on- and off-cells relative set points did not reveal statistical differences ($P = 0.30$ and $0.24$, respectively). If one considers the other variables recorded in rats maintained in thermoneural condition, the two groups of experiments were comparable.
of MAP, seen in all experiments whether during recordings of on- or off-cells. To compare the results related to on- and off-cells on a chronological ground, we decided to adjust the origin of time to these events. The occurrence of such events was detected by marking out the maximal negative slopes of the derivative dMAP/dt, witness of the fastest MAP drops. Individual episodes were collected that began 3 min before and ended 6 min following such a realigned origin of time. The global result of such an approach is shown in Fig. 8, A and B, for the on- and off-cells, respectively. Note: 1) the suddenness of the drop of MAP, as expected; 2) the resemblance of time course for the MAP and HR curves; 3) the resemblance of MAP and firing curves of off-cells; 4) the opposite image of the firing of on-cells; and 5) the ending of Tcore increase, 3 min or so following either the increase of on-cells firing or the decrease of off-cells firing (vertical arrows). Figure 8C summarizes the overall relationship between the cells firing and the variations of MAP. A strong linear relationship was obvious in the case of the off-cells. Roughly, a five spikes/s change of firing was synchronous to a 10 mmHg MAP variation. The picture was quite different in the case of the on-cells. A linear relationship was seen during the short duration (~1 min) ascending phase of firing; roughly, a five spikes/s rise of firing triggered a 10-mmHg MAP drop. This was followed by a long-lasting hysteresis return towards control values (dotted black arrow). Similar observations could be made regarding the overall relationship between the cells firing and the variations of HR (Fig. 8D).

Synthesis

An attempt to summarize our observations is provided in Fig. 9, which illustrates a single run of the core body temperature regulation (Fig. 9A). The on-cells firing (Fig. 9B) began when the core body temperature achieved an upper relative set point [red point on Tcore = f(t) curve]. Such firing was concomitant with a marked drop of the off-cells firing (Fig. 9C) and a fall in MAP, followed by HR drop within <1 min (Fig. 9D), an increase of Tskin (Tpaw and/or Ttail) within 2–3 min (Fig. 9E), and a decrease of Tcore within ~5 min (Fig. 9F). This period was followed by a transitional period of variable duration (red hatched area) during which the Tcore declines slowly as a consequence of both HR deceleration and peripheral vasodilatation. The opposite events happened when the core body temperature achieved a lower relative set point [blue point on Tcore = f(t) curve]: an increase in MAP, followed by HR acceleration within <1 min, was concomitant to a silence of on-cell firing and a rise of off-cell firing. These events were followed by a fall of Tskin within ~2–3 min and a rise of Tcore within ~5 min. This period was followed by a transitional period of variable duration (blue hatched area) during which Tcore increased slowly as a consequence of both HR acceleration and peripheral vasconstriction. When the Tcore achieved a new upper relative set point, the cycle reiterated.

Such ideal cycling occurred in thermoneutral conditions achieved by equilibrium between production and dissipation of heat. Note that a precise experimental determination of chronology was only possible around the fast blood pressure drops. The remaining chronology was estimated (see Table 2 in italics) on the basis of cross-correlation analyses between variables over the total duration of each individual experiments. This duration included the changeable periods shown as hatched areas in Fig. 9, marked out by the reversal of Tcore trend and the impending set point. These periods were essentially variable because the delay between Tcore downfall and impending lower set point, or Tcore rising and impending upper set point, depends on several factors, including, among others, the kinetic of Tskin cooling down or warming and set points variations.

![Fig. 7: A cumulative frequency histogram of results concerning 17 sequences of stable fluctuations of on-cell recording experiments in rats held in thermoneutral conditions.](http://jn.physiology.org/)

**Table 2:**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Power (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLF</td>
<td>0.0003</td>
</tr>
<tr>
<td>HF</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Fig. 7. A: cumulative frequency histogram of results concerning 17 sequences of stable fluctuations of on-cell recording experiments in rats held in thermoneutral conditions. Abscissa: logarithmic scale of frequencies in Hz. Ordinate: cumulative frequency histogram expressed in percentage of results namely on-cells (filled pink), MAP (red), HR (violet), Ttail-mid (blue), Tpaw-right (yellow), and Tcore (grey). Results were obtained from Fast Fourier Transform, performed on each signal when fluctuations were present (especially for tail and hind paws). A frequency domain was delimited in the 0.0003- to 0.006-Hz range with a peak between 0.001 and 0.003 Hz for all signals. Additional peaks in the 0.15- to 0.5-Hz range can be seen for MAP and HR. These belong to the low frequency (LF) domain, as defined in the MAP/HR variability literature (Julien et al. 2008; Persson et al. 1992; Stauß 2007). For facilitating the comparisons with this literature, we colored the background for very low frequency (VLF) and LF domains in orange and yellow, respectively. B: corresponding histogram of results concerning 15 sequences of stable fluctuations of off-cell recording experiments (identical presentation except off-cells shown as filled blue). Note that the frequency domain is delimited in the 0.0003- to 0.006-Hz range with a peak between 0.001 and 0.002 Hz for all signals. Comments as in A for MAP and HR peaks. C: graphic representation of the time laggins between MAP and the other variables, based on Table 2 data, namely on-cells, off-cells, HR, Tpaw-right, Ttail-mid, and Tcore. Code of color, please refer to Fig. 3.

![A on-cells experiments](http://jn.physiology.org/)  
![B off-cells experiments](http://jn.physiology.org/)  
![C time lag with MAP](http://jn.physiology.org/)
Table 1. Correlations between different signals during on-cell recordings in thermoneutral conditions

<table>
<thead>
<tr>
<th></th>
<th>On-Cell</th>
<th>MAP</th>
<th>HR</th>
<th>Tpaw-right</th>
<th>Tail-mid</th>
<th>Tcore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal Pearson’s correlation coefficients</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>On-cell</td>
<td>1</td>
<td>-0.71</td>
<td>-0.66</td>
<td>0.60</td>
<td>0.61</td>
<td>-0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.63–0.78)</td>
<td>(0.59–0.74)</td>
<td>(0.49–0.71)</td>
<td>(0.53–0.70)</td>
<td>(0.53–0.71)</td>
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<tr>
<td>MAP</td>
<td>1</td>
<td>0.73</td>
<td>-0.72</td>
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<td>0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.61–0.86)</td>
<td>(0.61–0.84)</td>
<td>(0.56–0.77)</td>
<td>(0.38–0.78)</td>
<td></td>
</tr>
<tr>
<td>HR</td>
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<td>-0.69</td>
<td>0.76</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(0.46–0.75)</td>
<td>(0.58–0.80)</td>
<td></td>
<td>(0.69–0.82)</td>
<td></td>
</tr>
<tr>
<td>Tpaw-right</td>
<td>1</td>
<td></td>
<td>0.72</td>
<td>-0.68</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td>(0.62–0.81)</td>
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<td></td>
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<tr>
<td>Tail-mid</td>
<td>1</td>
<td></td>
<td></td>
<td>-0.74</td>
<td>-0.66–0.82</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>(0–0.66–0.82)</td>
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<tr>
<td>Tcore</td>
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<td>31</td>
<td>55</td>
<td>173</td>
<td>214</td>
<td>352</td>
</tr>
<tr>
<td>Time lagging, s</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On-cell</td>
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<td>34</td>
<td>120</td>
<td>156</td>
<td>253</td>
<td>318</td>
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<tr>
<td></td>
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<td>(70–169)</td>
<td>(92–220)</td>
<td>(234–402)</td>
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</tr>
<tr>
<td>MAP</td>
<td>0</td>
<td></td>
<td>115</td>
<td>126</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(77–153)</td>
<td>(67–186)</td>
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</tr>
<tr>
<td>HR</td>
<td>4</td>
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<td>0</td>
<td>(28–37)</td>
<td>100</td>
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<td></td>
<td></td>
<td>(37–162)</td>
<td></td>
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<tr>
<td>Tpaw-right</td>
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<td>0</td>
<td></td>
<td>117</td>
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<tr>
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<td></td>
<td></td>
<td>(86–149)</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Tcore</td>
<td>0.66</td>
<td>0.60</td>
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<td></td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>(0.46–0.75)</td>
<td>(0.59–0.74)</td>
<td>(0.61–0.86)</td>
<td>(0.71–0.82)</td>
<td></td>
</tr>
</tbody>
</table>

Cross-correlations results between different variables [mean (95% confidence interval)] in anesthetized rat maintained in thermoneutral conditions. The relative delay at which maximum correlation was observed was used to estimate the time lag between 2 variables. The positive maximal correlation values are related to signals in phase and negative maximal correlations for signals in opposition of phase. Note that the maximal Pearson’s correlation coefficients were always >0.5 (or lower than –0.5), indicating that the variables were strongly correlated. Abbreviations: see glossary.

Consequence for the Study of Pain

We proposed and verified experimentally a simple model for computing the tail-flick latency (TFL) elicited by radiant heat in the rat, taking into account the power of the radiant heat source, the initial skin temperature, the core body temperature, and the site of stimulation on the tail (Benoist et al. 2008). This approach was successfully applied to the reconstruction of the tail-flick responses following a conditioned stress response (Carrive et al. 2011). This model was used here to compute the predictable variations of the TFL introduced by the spontaneous variations of the skin and core body temperatures seen in thermoneutral conditions. The variations of the TFL are inversely correlated to the variations of the temperature of the skin, the slope of this curve depending on the power of the radiant heat source. If one considers the individual examples provided in Figs. 3 and 5 and a site of stimulation on the mid-tail, the corresponding computed TFL would vary in the 2.4- to 3.6-s and 3.5- to 5.0-s ranges, respectively, for a power of the radiant heat source representative of the most commonly used procedures (slope of the squared temperature variation = 70°C2/s). The temporal evolution of the reconstructed TFL is shown in Fig. 10 together with MAP and the firing of the on- (Fig. 10A) and off-cell (Fig. 10B). Note 1) the covariations of MAP and the postponed TFL in both cases, 2) the inverse variations of these variables with the on-cell firing (Fig. 10A), and 3) the covariation of these variables with the off-cell firing (Fig. 10B). Clearly, the higher and lower values of TFL all occur during on-cell firing (grey background) and silence (white background), respectively. The converse situation is seen for the off-cell.

DISCUSSION

In a thermoneutral state where Tcore evolves cyclically within a narrow range of a few tens of degrees (El Bitar et al. 2014), the recordings were characterized by slow regular periodic changes of the variables at a recurrence of approximately three to seven cycles/h, organized in a chronological order as follows: core body temperature → on-cell → off-cell → blood pressure → heart rate → skin temperature → core body temperature. When, during a cycle, a relative high Tcore was reached, the on-cells were activated and within half a min, the off-cells and blood pressure were depressed, followed by HR within a further minute; vasodilation of the tail followed invariably within ~3 min, often completed with vasodilation of hind paws. The outcome was an increased heat loss that lessened Tcore. When Tcore decreased a few tenths of degrees, converse phenomena were seen: on-cells silencing, off-cells activation, blood pressure and HR increase, vasoconstriction of the tail and hind paws within ~3 min, and increase of Tcore providing a complete cycle. In fact, the drop or rise of blood pressure was followed by a variation of Tcore within ~5 min. On- and off-cell activities were linked to sympathetic inhibition and activation, respectively.

Of note, the regular alternating pattern of on- and off-cell activities seen in thermoneutral conditions can be interrupted following minute disturbances (~1°C) of the surrounding (cooling or warming) towards a new level of temperature. It happened concomitantly with disruption of the MAP, HR, and vasomotor tone regular patterns. These deviations were out of the topic of the present article.
The “Spontaneous” Variations of Activity

Baseline firing varied in the ~0- to 30-Hz range, but the overall mean baseline was lower for on- than for off-cells, probably because the on-cell were characterized by long periods of silence, as previously described (Leung and Mason 1998; Winkler et al. 2006). The simultaneous recordings of the spontaneous activities of pairs of neurons demonstrated: 1) the inverse relationship of on- and off-cells firing, such that a decrease in activity of cells of one class is accompanied by an increase in activity of cells of the other class, and 2) the similar fluctuations of all neurons of a given class (Barbaro et al. 1989). In fact, these variations are synchronized to the blood pressure variations.

Actually, our data confirm earlier reports showing that the spontaneous fluctuations in activity of on- and off-cells are negatively and positively correlated with MAP, respectively (Thurston and Randich 1992, 1995). We used spectral analyses to determine the frequency domains common to the physiological variables, notably MAP, and the activity of RVM/rMR neurons: the prominent peaks in the power spectra were in the 0.001- to 0.002-Hz range for all signals, that matches exactly the mean 0.0016 Hz described by Leung and Mason (1996), indicating mean fluctuations approximately six to seven cycles/h. Such frequencies belong to the “extremely low frequencies” (0.0006 – 0.021 Hz) recognized in studies related to blood pressure or HR variability (Julien et al. 2008; Persson et al. 1989). In fact, these variations are synchronized to the blood pressure variations.

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Again in keeping with the above-mentioned studies (Leung and Mason 1996; Thurston and Randich 1992, 1995), the spontaneous changes of on- and off-cell activity occurred before changes in MAP. Time lags were calculated from the cross-correlation analyses as the relative delay at which maximum correlation was observed to estimate the time lag between 2 variables. The positive maximal correlation values are related to signals in phase, and negative maximal correlations for signals in opposition of phase. Note that the maximal Pearson’s correlation coefficients were always ≥0.5 (or lower than –0.5), indicating that the variables were strongly correlated. Comparisons with time lags observed in the on-cells series of experiments (see Table 1) are added in italics. The only significant difference was seen for MAP. The other variables were therefore grouped in the lower line of the table where lags with MAP are summarized. N.S., nonsignificant; other abbreviations: see glossary.
Fig. 8. Synchronization of experiments related to on- and off-cells. The origin of time was adjusted to the sudden drops of arterial blood pressure, detected by marking out the maximal negative slopes of the derivative \( dMAP/dt \).

Individual episodes began 3 min before and ended 6 min following such a realigned origin of time with the first 2-min period being taken as control period (grey background). Individual results were presented as difference to the mean of this control period. The means of such recordings were considered as representative of each cell and grand means were calculated.

**A**: temporal evolution of MAP, HR, \( T_{\text{core}} \), and firing of on-cells. **B**: Temporal evolution of MAP, HR, \( T_{\text{core}} \), and firing of off-cells. Abscissa: time in seconds. **Left ordinate**: mmHg for MAP variation; beats/min for HR variation and spikes/s for cells firing variation. **Right ordinate**: °C for \( T_{\text{core}} \) variation. The downward pointing arrow indicates the ending of \( T_{\text{core}} \) increase.

**C**: relationship between the cells firing and the variations of arterial blood pressure. Abscissa: spikes/second for cells firing variation. Ordinate: mmHg for MAP variation. The linear relationship seen during the short duration ascending phase of firing: roughly (dark pink) was followed (light pink) by a long-lasting hysteresis return towards control values (dotted black arrow).

**D**: relationship between the cells firing and the variations of heart rate. Abscissa: spikes/s for cells firing variation. Ordinate: beats/min for HR variation (see text).
increase in core body temperature and decrease in shell temperature and vice versa, successively. They constitute apparently conflicting inputs to medial preoptic area neurons. However, the influence of $T_{\text{core}}$ was clearly preponderant in thermoneutral states. However, a small variation of the ambient or warming temperature, in the tenths of Celsius degree range, could disrupt such dynamic equilibrium. In these cases, one can postulate that the fragile balance breaks in favor of inputs from the shell constitutes a feed-forward mechanism (Kanosue et al. 2010). Such an interpretation is supported by the fact that both skin and core body temperatures are important determinants of tail sympathetic fiber activity (Owens et al. 2002; Sakurada et al. 1993; Young and Dawson 1982). The mechanism that performs this regulation remains to be determined.

The Responses to Radiant Heat

The responses of RVM/rMR neurons to radiant heat applied to the tail were similar to those reported by Fields and colleagues, that is preceding the occurrence of the tail-flick. However, both acceleration for on-cells and deceleration for off-cells began $\sim$2.5 s before the occurrence of the tail-flick. This differed slightly with their reports suggesting that the on- and off-cell responses occur 0.5 and 0.7 s before the tail-flick (Fields et al. 1983; Hernandez et al. 1989) but are closer to the 2 s reported by Thurston and colleagues (Thurston and Helton 1996; Thurston and Randich 1992, 1995). We also observed that the drop of the off-cell firing reached 50% of the total change before the rise of on-cell firing did reach 50% of the peak, in accordance with Cleary et al. (2008). Both peaks occurred 0.4–0.5 s following the end of the stimulation, itself produced by the withdrawal of the tail. Such a delay is what one can expect from the “latency artifact,” that is the time required for the response to occur when the threshold is achieved, because we determined on a psychophysical ground that the true behavioral latency was always $<0.5$ s, depending on the temperature of the skin (Benoist et al. 2008). Leung and Mason (1998) used a Peltier device taped on the tail and concluded, at variance with the other authors, that the bulk of the change in on- and off-cell discharge evoked by tail heat occurred after the onset of the motor response and even after the conclusion of the visible motor response, probably because the heating was not stopped by the response.

As others (Thurston and Helton 1996; Thurston and Randich 1992, 1995), we also noticed a drop of MAP peaking within 2–3 s following the withdrawal responses. The heat-evoked changes in on- and off-cell discharge were therefore clearly not secondary to the autonomic responses.

The Responses to Thermal Challenge

Thermoresponsive neurons were recorded in the RVM/rMR that were not affected by noxious stimuli (Dickenson 1977; Hellon and Taylor 1982). By contrast, Young and Dawson (1987) observed that cold- and warm-responsive RVM/rMR neurons were excited and inhibited by noxious mechanical or thermal stimuli, respectively. In this case however, the stimuli, notably the cold, were strong indeed. These neurons were assumed to form part of an ascending pathway to the hypothalamus and thalamus (Berner et al. 1999; Taylor 1982), and one cannot exclude the possible existence of ascending collateral connections in addition to descending pathways. However,
Fig. 10. Temporal evolution of the predictable TFL. We proposed and verified experimentally a simple model for computing the TFL in the rat, taking into account the power of the radiant heat source, the initial temperature of the skin, the core body temperature or the site of stimulation on the tail (Benoist et al. 2008). This model is used here to compute the predictable variations of the TFL introduced by the spontaneous variations of the skin and core body temperatures seen in thermoneutral conditions. Considering a site of stimulation on the mid-tail far-off the dorsal horn entry zone by 200 mm, the model provides the following equation giving the tail-flick latency: TFL (s) = [(36.8 – 0.73 × T(on))2/α + 90(0.041 × T(core) – 0.47) + 110(0.041 × T(mid) – 0.47) + 138]/1,000 where α is the slope of the squared temperature variation, witness of the power of the radiant heat source. Experimental data are from the individual examples of on-cell (A) and off-cell (B) provided in Figs. 3 and 5 and α = 0.07°C/ms. The model foretells an inverse correlation between the variations of the temperature of the skin and the variations of the TFL. The lag between the variations of the cell firing and the variations of T(mid) was 6.6 and 4.2 min for the on- and off-cell, respectively. The corresponding MAPs are also introduced. Abscissa: time in min. Left blue ordinate: computed TFL (s). Right black ordinate: cell firing (spikes/s). Right red ordinate: MAP (mmHg). Note that the higher and lower values of TFL all occur during on-cell firing (grey background) and silence (white background), respectively. The converse situation is seen for the off-cell.

Rathner et al. (2001) reported that most RVM/rMR neurons, both antidromically activated from the lumbar DLF and activated by mild cold, also responded to noxious tail pinch, most being inhibited (possibly off-cells), but some being excited (possibly on-cells). The comparison of these observations with our data is limited because 1) they were obtained under anesthesia of sufficient depth to abolish withdrawal reflexes; 2) the search stimuli were cyclic variations of the temperature inflicted on the trunk of the animal; and 3) these temperature variations were large.

In keeping with the pain-related literature, our recordings were made under an anesthetic regime that preserved withdrawal reflexes and cells were characterized by their ability to respond to noxious heat. The temperature of the trunk remained stable and the trigger of cell firing was found related to the spontaneous variations of the core body temperature. When we warmed slightly the room or the temperature surrounding the trunk of the rat, we observed 1) hyperactivity of on-cells and silencing of off-cells; 2) fading of blood pressure and HR variability; 3) peripheral vasodilatation; and 4) decrease of T(core). Following slight cooling, we observed: 1) hyperactivity of off-cells and silencing of on-cells; 2) increase of the frequencies of blood pressure and HR variability; 3) peripheral vasoconstriction; and 4) increase of T(core). The off-cells might then correspond to the neurons activated by mild cold, as in the recordings of Rathner et al. (2001). However, one should not forget the time lag (~5 min) between the variations of cellular firing and the variations of the central temperature that could confuse the issue when the experimental condition moves away from thermoneutrality. The hypothesis that off-cells are cold responsive and on-cells warm responsive must be verified.

In any case, these considerations extend to thermal and cardiovascular information the non-nociceptive sources of RVM/rMR inputs. Indeed, such neurons could be excited or inhibited to the same degree by noxious heat and very light touch in both anesthetized (Leung and Mason 1999; Thurston and Randich 1992, 1995) and nonanesthetized (Leung and Mason 1999; Olivéras et al. 1989, 1990) animals. In addition, they respond to auditory stimulation in the non-anesthetized rat (Leung and Mason 1999; Olivéras et al. 1989, 1990). Although on- and off-cells do respond to noxious mechanical stimuli in a manner similar to their response to noxious heat (Leung and Mason 1999), these observations provide some doubts regarding the claimed specific nociceptive features of on- and off-cells recorded in the RVM/rMR (Fields et al. 2006).

Consequences for Pain Studies

The reaction time measured following conventional progressive heating, as in the conventional tail-flick test, is the sum of 1) the time to achieve the threshold for the behavioral reaction and 2) the behavioral latency. We proposed and verified experimentally a simple model for computing the TFL, taking into account the power of the radiant heat source, the initial temperature of the skin, the core body temperature, and the site of stimulation on the tail (Benoist et al. 2008; Pincedé et al. 2012). This is not the place to discuss this model, but we used it to compute the predictable variations of the TFL introduced by the cyclic vasomotor variations. They were inversely correlated to the temperature of the skin of the tail and, therefore, roughly correlated to the blood pressure and HR variations but postponed by several minutes (Fig. 10). Accordingly, positive and negative correlations between TFL and on- and off-cell firing, respectively, are expected and were de facto already described (Heinricher et al. 1989).
Both descending facilitatory and inhibitory influences on the tail-flick reflex were reported following RVM/rMR stimulation (Gebhart 2004; Zhuo and Gebhart 1997). Facilitations or inhibitions were seen with low or higher (≥50 μA) intensities of electrical stimulation or low or higher (≥50 nmol) concentrations of glutamate microinjection, respectively. Roughly, electrical stimulation in RVM/rMR only facilitated the tail-flick reflex in 10–15% of cases. The remaining cases divide up in an equal way in inhibitory and biphasic (intensity-dependent facilitation and inhibition from the same site) effects. Since on-off-cells are anatomically intermixed, it was not clear why some sites were “pro-” while other were “antinociceptive.” In any case, the main point here is the fact that the facilitation drive was conveyed through ventral/ventrolateral funiculi, while the inhibition drive was conveyed through the DLF. Unfortunately, the cardiovascular effects of the former were not described but the latter was reported to increase the arterial blood pressure. This makes sense because the sympathetic premotor neurons in the RVM/rMR project to the intermediolateral column through the DLF (Bacon et al. 1990; Loewy 1981; Morrison 1993; Morrison and Gebber 1985; Nalivaiko and Blessing 2002; Rathner et al. 2001; Smith et al. 1998; Stornetta et al. 2005; Strack et al. 1989). Taking into account the autonomic-related literature led to the inescapable conclusion that the “antinociceptive” stimulations both increased arterial blood pressure and produced peripheral vasoconstriction.

That RVM/rMR is involved in the control of sympathetic outflow is well known (see references in Barman 1990; Blessing 2003; Dampney 1994; McAllen et al. 2010; Morrison 2011; Morrison and Blessing 2011; Morrison and Nakamura 2011; Nakamura 2011; Nakamura and Morrison 2008). For example, electrical or chemical stimulation in RVM/rMR can evoke both pressor and depressor responses in the cat. Similarly, both sympatho-excitatory and sympatho-inhibitory responses can be recorded in sympathetic nerves following such stimulation (Adair et al. 1977; Henry and Calaresu 1974; McCall 1984; Morrison and Gebber 1982; Rathner and McAllen 1999; Yen et al. 1983). In the rat, experimental activation of the RVM/rMR, e.g., by microinjection of bicuculline or glutamate receptor agonists, evokes sympatho-excitation including tachypnea, increase of blood pressure, tachycardia, increase activity of brown adipose tissue, and peripheral vasoconstriction (Blessing 2003; Blessing and Nalivaiko 2000, 2001; Cao and Morrison 2003; Madden and Morrison 2003; Morrison 1999, 2001; Nason and Mason 2004; Ootsuka and McAllen 2005; Rathner and McAllen 1999; Zaretsky et al. 2003). Note, however, that microinjection of glutamate in the RVM/rMR was also reported to evoke bradycardia, fall in arterial blood pressure, and apnea (Minson et al. 1987; Verner et al. 2004).

Conversely, lesions of RVM/rMR abolish the decrease in blood pressure and inhibition of sympathetic nerve activity elicited by electrical stimulation of the spinal trigeminal tract (McCall and Harris 1987). In the rat, functional blockade of RVM/rMR neurons, e.g., by microinjection of glycine, GABA, or muscimol, freezes the sympathetic fibers that innervate the tail or the hind paws (Blessing and Nalivaiko 2001; Cerri et al. 2010; Korsak and Gilbey 2004; Ootsuka et al. 2004; Ootsuka and McAllen 2005; Vianna et al. 2008).

These observations parallel several studies from the “pain field.” Administered within RVM/rMR, bicuculline or muscimol increases or decreases, respectively, the behavioral latencies in the tail-flick, paw-withdrawal, and hot-plate tests (Drower and Hammond 1988; Gilbert and Franklin 2001; Heinricher and Kaplan 1991; Heinricher and Tortorici 1994; Martensen et al. 2009; Nason and Mason 2004). Also, spontaneous or induced hypertension was repeatedly reported to increase the reaction time in the tail-flick and hot plate tests (Afolabi et al. 2013; Ghione 1996; Hoffmann et al. 1998; Maixner et al. 1982; Maixner 1991; Randich 1982; Randich and Gebhart 1992; Randich and Maixner 1984; Saavedra 1981; Sitzen and de Jong 1983; Sitzen and Nijkamp 1984; Taylor et al. 2001; Tchakarov et al. 1985; Thurston and Randich 1990; Zamir and Maixner 1986). Reviewing data suggesting interactions between cardiovascular and pain systems, Randich and Maixner (1984) noticed the possibility of convergence of information to RVM/rMR that can affect both withdrawal of sympathetic tone through inhibition of preganglionic sympathetic neurons in the intermediolateral cell columns and inhibition of nociceptive input from receptive fields in the tail. This notion is now clarified, as discussed above.

However, by considering the relationship described here between MAP/HR and the vasomotion of the tail and paws, then a first fundamental explanation, which does not exclude any other additional factors of variation, is to be found regarding the relationship between BP/HR and the temperature of the skin. The TFL is inversely correlated to the temperature of the tail, itself inversely correlated to the arterial blood pressure. The TFL is therefore correlated to the blood pressure and HR variations but slightly postponed.

Summaries and Conclusions

We described a possible involvement of RVM/rMR on-and off-cells in the preservation of the core body temperature in a narrow range, therefore, participating in the homeostasis required over the whole life time (Bernard 1865; Cannon 1932). On a long-term basis, such basic balance must be preserved but can be broken by many circumstances involving internal and external factors generated by everyday life (e.g., circadian variations of temperature, mild stress, salient stimuli) or more dramatic situations (e.g., strong stress, illness, pain).

According to Mason (2005b), knowing the entirety of RVM/rMR’s projections excludes the mediation of these structures to any specific, including homeostatic, function. It is likely that they modulate several, e.g., cardiorespiratory, thermoregulatory, and nociceptive, functions (Nason and Mason 2004). In particular, they are involved in the regulation of cutaneous blood flow that occurs as part of body temperature control and as part of emotionally mediated cutaneous vasoconstriction elicited by salient, including nociceptive and stressful, stimuli (Blessing 2003). Clearly, blood is diverted from the skin to other parts of the body when the animal detects a salient threatening stimulus (de Menezes et al. 2009; Mohammed et al. 2013). For example, a sudden unexpected noise does cause a prompt and vigorous vasoconstriction in the tail bed but not the mesenteric bed of conscious rats (Garcia et al. 2001). In fact, these phenomena are the object of three different sources of literatures, namely “stress-induced hypertension,” “stress-in-
duced hyperthermia,” and “stress-induced analgesia” that we believe are profoundly coupled.

ACKNOWLEDGMENTS

We thank François Cesselin and Léon Plaghi for advice in the preparation of the manuscript.

GRANTS

N. El Bitar was supported by a grant from the Société Française d’Étude et de Traitement de la Douleur (SFETD) et l’Institut UPSA de la Douleur (IUD).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.E.B., B.P., and D.L.B. conception and design of research; N.E.B. and B.P. performed experiments; N.E.B., B.P., and D.L.B. analyzed data; N.E.B., B.P., and D.L.B. interpreted results of experiments; N.E.B. and D.L.B. drafted manuscript; N.E.B., B.P., and D.L.B. edited and revised manuscript; N.E.B., B.P., and D.L.B. approved final version of manuscript.

REFERENCES


Afzal AO, Mudashiru SK, Alagbon SI. Effects of salt-loading hyperten-

Bacon SJ, Zagon A, Smith AD. Electron microscopic evidence of a mono-


Basbaum AI, Fields HL. The origin of descending pathways in the dorsolat-


Blessing WW. Lower brainstem pathways regulating sympathetically medi-


Covino BG, Dubner R, Gybels J, Kosteritz HW, Liebeskind JC, Stern-
brach RA, Vyklický L, Yamamura H, Zimmermann M. Ethical stan-


de Menezes RC, Otsuka Y, Blessing WW. Sympathetic cutaneous vasomo-


physiol. First published July 9, 2014; doi:10.1152/jn.00721.2013.


Fields HL, Basbaum AI. Central nervous system mechanisms of pain mod-

Fields HL, Basbaum AI, Heinricher MM. Central nervous system mecha-


Heinricher MM, Tortorici V. Interference with GABA transmission in the rostral ventromedial medulla: disinhibition of off-cells as a central mecha-


Mason P. From descending pain modulation to obesity via the medullary raphe. Pain 152: S20–S24, 2011.


Sitsen JM, de Jong W. Hypoalgesia in genetically hypertensive rats (SHR) is absent in rats with experimental hypertension. *Hypertension* 5: 185–190, 1983.


Winkler CW, Hermes SM, Chavkin CI, Drake CT, Morrison SF, Aicher SA. Kappa opioid receptor (KOR) and GAD67 immunoreactivity are found in OFF and NEUTRAL cells in the rostral ventromedial medulla. *J Neurophysiol* 96: 3465–3473, 2006.


