Changes in cutaneous and body temperature during and after conditioned fear to context in the rat

Author/Contributor:
Vianna, Daniel; Carrive, Pascal

Publication details:
European Journal of Neuroscience
v. 21
Chapter No. 9
pp. 2505-2512

Publication Date:
2005

Publisher DOI:
http://dx.doi.org/10.1111/j.1460-9568.2005.04073.x

License:
https://creativecommons.org/licenses/by-nc-nd/3.0/au/
Link to license to see what you are allowed to do with this resource.

Downloaded from http://hdl.handle.net/1959.4/38911 in https://unswworks.unsw.edu.au on 2023-09-15
CHANGES IN CUTANEOUS AND BODY TEMPERATURE DURING AND AFTER CONDITIONED FEAR TO CONTEXT IN THE RAT.

Daniel M L Vianna and Pascal Carrive

School of Medical Sciences, University of New South Wales, NSW 2052, Australia

Running title: Changes in skin temperature during fear

Total number of pages: 22
Total number of figures: 3
Total number of words: 6339
Number of words in Abstract: 251
Number of words in Introduction: 552
Key words: Stress, thermoregulation, sympathetic, blood flow, tail

Address for correspondence:
Pascal Carrive
School of Medical Sciences, University of New South Wales, NSW 2052, Australia
Email: p.carrive@unsw.edu.au
Fax: 61 2 9385 8016
ABSTRACT

Infrared thermography was used to image changes in cutaneous temperature during a conditioned fear response to context. Changes in heart rate, arterial pressure, activity and body (intraperitoneal) temperature were recorded at the same time by radio-telemetry, in addition to freezing immobility. A marked drop in tail and paws temperature (-5.3 and -7.5°C respectively -- down to room temperature), which lasted for the entire duration of the response (30 min), was observed in fear conditioned rats. In sham conditioned rats, the drop was on average half the magnitude and duration. In contrast, temperature of the eye, head and back increased (between +0.8 and +1.5°C) with no difference between the two groups of rats. There was a comparable increase in body temperature although it was slightly higher and delayed in the fear conditioned animals. Finally, ending of the fear response was associated with a gradual decrease in body temperature and a rebound increase in the temperature of the tail (+3.3°C above baseline). This study shows that fear, and to some extent arousal, evokes a strong cutaneous vasoconstriction that is restricted to the tail and paws. This regionally specific reduction in blood flow may be part of a preparatory response to a possible fight and flight to reduce blood loss in the most exposed parts of the rat’s body in case of injury. The data also show that the tail is the main part of the body used for dissipating internal heat accumulated during fear once the animal has returned to a safe environment.
INTRODUCTION

The fear reaction brings up a set of behavioural and autonomic changes that help the organism respond to potential external threats to its survival. In rats, the most obvious of these changes is seen in behaviour. Rats freeze, that is, they assume an immobile posture that helps concealment, but at the same time, their body prepares internally for an active defence response such as fight or flight (Blanchard et al., 1986; Walker & Carrive, 2003). Cardiovascular changes are a good example: heart rate increases and the visceral vasculature constricts, presumably to increase perfusion pressure and redirect blood flow to where it may be most needed, the skeletal musculature. Vascular changes can also occur in other parts of the body, and in particular in the skin. It is well known that frightening stimuli in human can cause palor, usually recognized in the face. This is due to a decrease in skin blood flow, which also causes cooling of the skin. Consistent with this, Blessing and collaborators have shown that alerting environmental stimuli can cause profound reductions in blood flow in the rabbit’s ear and in the rat’s tail, two territories that are mainly cutaneous (Blessing, 2003; Blessing & Seaman, 2003; Nalivaiko & Blessing, 1999; Garcia et al., 2001; Yu & Blessing, 1997, 2001).

To our knowledge, changes in skin blood flow during fear have never been investigated before in the rat. From the work of Blessing and collaborators, one would expect vasoconstriction in the tail, however a more important question is whether skin vasoconstriction is restricted to the tail or whether it occurs all over the body. In the rabbit, Nalivaiko & Blessing (1999) have shown that alerting stimuli cause vasoconstriction in both the ear and tail, which led them to suggest that skin vasoconstriction might be generalized rather than localized. Unfortunately this cannot be tested easily because of the difficulty to record skin blood flow in multiple parts of the body at the same time, especially in the conscious animal. To circumvent this problem we thought of using infrared thermography to image and record an indirect measure of skin blood flow, skin temperature. Skin temperature, which varies between room temperature (25°C)
and skin blood temperature (approximately 34 °C) is considered a good index of skin blood flow as long as the ambient temperature is held constant (Felder et al, 1954; Hertzman, 1953). Skin temperature can in turn be assessed remotely by infrared thermography from the amount of heat it radiates in the infrared spectrum (van den Heuvel et al., 2003; Jones & Plassmann, 2002). Infrared thermographic cameras are now available that have sufficient resolution and sensitivity to reliably record surface temperature in visible parts of the body, and with the added advantage that the recording does not interfere with the behaviour of the animal (Anbar, 2002; Kastberger & Stachl, 2003; Head & Elliott, 2002).

The aim of the present study was to use this new technology to image and measure the regional changes in skin temperature that occur in the rat during conditioned fear to context, a well known laboratory paradigm which we have used already (Carrive, 2000, 2002; Walker & Carrive, 2003). These changes were compared to freezing behaviour and changes in body temperature, mean arterial pressure, heart rate and activity recorded at the same time by radio-telemetry.

MATERIALS AND METHODS

The subjects were 12 experimentally naive male Wistar rats (350-500g) obtained from the colony of specific pathogen-free rats maintained by the University of New South Wales. The rats were kept in individual plastic home boxes (38 cm long x 25 cm wide x 60 cm tall, more explanation below) with ad libitum food and water throughout the experiment. The animals were housed in the same room during the entire duration of the experiment. It was maintained on a 12-h light/dark cycle and the experiments were conducted during the light phase of the cycle. The room was air conditioned and maintained at a constant temperature of 26-27 °C. All experiments were approved by the Animal Ethics Committee of the University of New South Wales and conformed to the rules and guidelines on animal experimentation in Australia.
Radio-telemetric probe implantation

Rats were implanted with Data Sciences International radio-telemetric probes for measurement of blood pressure, activity and body temperature (C50-PXT, \(n=10\)) or blood pressure and activity (PA-C40, \(n=2\)). The surgery was done in aseptic conditions and under anesthesia with a mixture of ketamine (Ketamil, 120 mg/Kg, i.p.) and xylazine (Ilium Xylazil-20, 6.5 mg/Kg, i.p.). The rats were also pretreated with the analgesic Carprofen (Rimadyl, 5 mg/Kg, s.c.) and received antibiotics (Benicillin, 0.3 ml, i.p.) at the end of surgery. The probes were implanted in the peritoneal cavity, with the catheter sitting in the descending aorta at the level of the iliac bifurcation, as previously described (Carrive, 2000). During the recovery period (1 week) the animals were handled every day to habituate to the experimenter.

Fear conditioning

Conditioning and testing were done in footshock chambers (23 cm long x 21 cm wide x 60 cm tall, more explanation below) made of clear Perspex walls on two sides with a grid floor composed of 18 stainless steel rods (2 mm in diameter), spaced 1.5 cm apart, and wired to a shock generator. The chambers were cleaned before and after use with 0.5% acetic acid. Conditioning \((n=6)\) started at least 1 week after probe implantation and consisted of three conditioning shock sessions done on separate days over a period of 4 days. Each shock session consisted of a 40-min long exposure to the footshock chamber with four unsignaled electric footshocks (1mA, 1s) delivered at approximately \(t = 5, 15, 25\) and 35 min. Control rats (Sham conditioned, \(n=6\)) were subjected to a similar procedure, with the exception that they never received footshocks.

Infrared imaging

Surface temperature of the rat and its immediate surrounding was recorded with an infrared digital thermographic camera (TVS-100, Avio, Japan) placed 70 cm above the animal. This camera has a thermal sensitivity of approximately 0.1 °C
and a spatial resolution of 320X240 pixels [for more information about thermographic cameras see Jones & Plassmann (2002) and Kastberger & Stachl (2003). As infrared radiations are blocked by Plexiglass or stainless steel, it was necessary to remove the lids of the home boxes and footshock chambers to allow recording. The lids were therefore replaced by four 60 cm-tall plexiglass walls permanently fitted on both the home cage and the footshock box. The walls were high enough to prevent escape by jumping.

Testing

Both fear conditioned and sham conditioned animals were tested by a 32 min re-exposure to the footshock chambers during which no shock was delivered. The procedure was as follows. First, radio-telemetric probes were turned on. After a few hours of baseline recording in the home box, the rats were gently removed and transferred in the same footshock chamber in which they had been fear or sham conditioned. At the end of the test re-exposure, the animals were returned to their home box. Telemetric recording was continued for at least another 90 min or until the animals had returned to rest. At the same time, infrared thermographic images of the rats were captured every 2 min starting in the home box 30-40 min before re-exposure to the shock chambers and extending for at least 1 hour after return to the home boxes. The 12 rats were tested on 3 separate days, four at a time.

Data collection and analysis

Six parameters were measured, all concurrently. Two were cardiovascular (mean arterial pressure [MAP] and heart rate [HR]), two were behavioural (activity and freezing) and two were thermal (body [intraperitoneal] and surface temperature). MAP and HR were extracted automatically from the pulsatile blood pressure signal recorded by the radio-telemetric probes, using the ART gold software (Data Sciences International) running on a PC computer. They were sampled every 15 s from 3-s time windows. Activity was a continuous measure of body movements, which was automatically extracted by the software from changes in
orientation of the probes. Body temperature was also automatically extracted every 15 s from those probes that had a thermistor inside (C50-PXT, n=10). All these parameters were later averaged over 1-min periods. In addition to those four automatic measures, an experimenter standing above the shock chambers took infrared pictures every 2 min, while another experimenter sitting in front of them recorded manually the time spent freezing. Freezing was sampled every 8 s and cumulated over 1 min periods. It was defined as a complete absence of movement while the animal assumed a characteristic tense posture. Infrared pictures were later analyzed with the Goratec PE Professional 3.12 software, and temperatures for each region of interest were taken from representative single pixel measurements. Since thermal values depend on the emissivity of the object being imaged (its propensity to radiate in the infrared spectrum), emissivity was set at 0.98 which is the emissivity of the skin (as listed in the operating manual supplied by the manufacturer). We also established that the emissivity of rat fur is in the same range as that of skin. This was checked with thermocouple electrodes in a dead rat after its temperature (body, fur and skin) had equalized to room temperature. The regions of interest were the tail (middle section) the paws (forelimb or hind limbs), the top of the head (at equidistance from the eyes and ears), and the back (1 cm off the midline at upper lumbar level). Depending on the posture of the animals some areas were sometimes concealed and could not always be imaged (eg paws and occasionally head).

Statistical Analysis

Data were finally analyzed with StatView 5 (SAS Institute Inc.) using either simple or one way repeated measure ANOVAs. The repeated measure was time, the independent factor for one way analysis was shock during conditioning and statistical significance was set at $p< 0.05$.

RESULTS
Figure 1 shows the average changes in MAP, HR, activity, freezing, body temperature (in the peritoneal cavity) and surface temperatures of the back and tail when fear conditioned and sham conditioned animals were re-exposed to the shock box. Animals were at rest in their home box prior to re-exposure and baseline values were approximately the same in the two groups (on average 101 mmHg, 338 bpm, 37.4 °C for body temperature, 31.9 °C for back temperature and 31.5 °C for tail temperature). Average HR and back temperature were slightly more elevated in the sham conditioned group (+ 18 bpm and + 0.25 °C), but this was not statistically significant [F(1,19)=2.18 and F(1,9)=1.16, respectively, p>0.17].

Changes in fear conditioned animals

Re-exposure of the fear conditioned animals to the aversive context of the shock box evoked the typical conditioned fear response as previously described (Carrive, 2000, 2002). Behaviourally, the response was characterized by a tense freezing immobility that lasted for practically the entire 30-min exposure. Return to the safe home box at the end of the re-exposure was associated with a 10 min burst of activity which was followed by a return to rest within 50-60 min. The cardiovascular response was characterized by an increase in MAP and HR. After a quick rise of +31 mmHg, MAP remained elevated for the rest of the re-exposure (on average +26 mmHg above baseline). HR did not rise as fast, but reached a peak of +100 bpm during the first 15 min and then stabilized (on average +80 bpm). Upon returning to the home box at the end of the re-exposure, HR showed a transient increase at the same time as the burst in activity and then both MAP and HR gradually returned to baseline within 50-60 min.

The fear response was also associated with an increase in body temperature [F(4, 28)= 44.66, p<0.0001], however the pattern of the response was different.
For the first 7 min of the re-exposure, body temperature did not rise but dropped from 37.45 to 37.24 °C (-0.19 °C). It then started to rise steadily, reaching 38.50 °C by the end of the re-exposure (+1.05 °C) to finally peak at 38.86 °C (+1.41 °C), 7 min after the end of the re-exposure. Thus, the duration of the rise in body temperature was the same as the re-exposure (30 min). The fact that it was the same 7 min delay at the beginning and end of the re-exposure is noteworthy and suggests that the response might have been shifted in time. Body temperature then gradually returned to baseline but at a slower rate than MAP, HR or Activity (i.e., baseline was reached 10-15 min later).

Re-exposure to the aversive context also increased the surface temperature of the back \( [F(5,14)=4.94, \ p<0.0001] \). By the end of the re-exposure or shortly thereafter it was +1.2 °C above baseline (from 31.7 to 32.9 °C) which was of the same order of magnitude as the increase in body temperature. This was followed by a quick return to baseline, within 30 min. Temperature continued to drop in the next 30 min and stabilized approximately 1 °C below baseline.

The changes in tail surface temperature were different and a lot more obvious. First, tail temperature was very variable, ranging between 26.6 and 35.4 °C during the 30 min period prior to re-exposure (see Fig 2). Then from an average of 31.6 ± 0.5 °C in the last 10 min preceding the re-exposure it dropped down to room temperature where it stabilized and remained until the rest of the re-exposure \( [F(5,14)=7.21, \ p<0.0001; \ Fig \ 1] \). The average temperature in the last 10 min of the re-exposure was 26.3 ± 0.3 °C, that is -5.3 °C from baseline. It was clear that the tail was at the same temperature as the environment around it, because it had virtually “disappeared” in the background of the infrared image (Fig 3). Return to the home box was followed by a rebound increase in tail temperature that peaked at 34.9 °C, i.e., +3.3 °C above baseline. It occurred 10 min after the end of the re-exposure at the same time that body temperature started to decline. The two temperatures then gradually came back towards baseline which they reached at approximately the same time.
As can be seen on Figure 3, the infrared camera also revealed the surface temperature of other parts of the body. Figure 2 shows the result of this more detailed analysis. We chose to display individual data rather than average values because some parts of the body (e.g., paws) were occasionally concealed, which prevented calculations of means at 2 min intervals (as well as the repeated measure statistical analysis). Nevertheless, enough data could be collected for front and hind paws combined to show that the pattern of fear-evoked changes in paw surface temperature was similar to that of the tail. Here also there was a sharp drop towards ambient temperature (from 34.8 to 27.3 °C, i.e., -7.5 °C) with no further change until the end of the re-exposure. The effect appeared more pronounced than for the tail because of the higher and less variable baseline temperature prior to re-exposure. The end of the re-exposure and return to the home box was marked by a return to baseline temperature which was reached within 15 min.

Data collected for the eye and top of the head show that the changes in surface temperature in these two regions were similar to those of the back (Figs 2 & 3). There was a steady increase in both regions, from 35.2 to 36.4 °C (+1.2 °C) for the eyes and from 32.8 to 34.3 °C (+1.5 °C) for the head [F(5,14)= 8.04 and F(5,14)= 31.99, respectively, p<0.0001]. Return to baseline for the head appeared to be as fast as for the back (30 min), but was faster for the eyes (14 min).

We also looked at temperature changes in the ear. This area is not represented on Figure 2 (but see Fig 3) because it was more difficult to measure due to the narrow edge of the ear when seen from a dorsal aspect. Nevertheless, we noted that the skin of the ear behaved very much like the skin of the head and back i.e. it warmed up by 1.2 to 1.5 °C but never got colder.

Comparison with sham conditioned animals

Figures 1, 2 and 3 also show the changes that occurred in sham conditioned
animals. Comparison with the fear conditioned animals reveals noticeable differences. As expected, the most significant difference was the absence of freezing during re-exposure to the shock box \([F(1,28)=96.82; \ p<0.0001]\). This can also be seen on the Activity trace (Fig 1). Sham conditioned animals were more active \([F(1,28)=13.90; \ p=0.0039]\) because they were exploring the box instead of freezing, especially during the first half of the re-exposure. The second most significant difference was the lower MAP response (peak +18 mmHg, average +12 mmHg) \([F(1,28)=41.39; \ p<0.0001]\). No difference in the HR response could be detected when the whole 30 min period was considered \([F(1,28)=1.08; \ p=0.32]\), however, HR in the first 10 min was significantly higher (peak of +120 mmHg) than in fear conditioned animals \([F(1,9)=6.16; \ p=0.03]\). Not surprisingly, this was also the period when these animals were most active.

Unlike fear conditioned animals, the body temperature of sham conditioned animals started rising from the beginning of the re-exposure (Fig 1). It reached a plateau at 38.37 °C (+0.92 °C from baseline) 20 min within the re-exposure period, remained at this level until 30 min after the end of the re-exposure and then gradually returned to baseline. Clearly, the rise in body temperature occurred principally during the first half of the re-exposure when animals were active. During this period, body temperature was also higher than in fear conditioned animals, although statistical analysis over the first 10 min, 15 min or 20 min of the re-exposure actually failed to reach significance \((F(1,9-19)<4.28, \ p>0.07)\).

A decrease in tail temperature was also observed in the sham conditioned rats, however, the drop was on average smaller and shorter than in the fear conditioned rats (Figs 1 & 2). From an average of 31.1± 1.12 °C in the last 10 min before re-exposure, it dropped to an average of 28.6 ± 1.5 °C between the 10th and 20th min (i,e -2.5 °C drop compared to –5.3 °C in fear conditioned animals). Most importantly, it was not maintained throughout the re-exposure. Thus in the last 10 mins of the re-exposure, tail temperature of most sham-conditioned animals was back to baseline or at least higher than in fear.
conditioned animals (4 of the 6 sham-conditioned animals in the last 10 min and 5 in the last 6 min). Only one sham conditioned rat had its tail as cold as the fear conditioned animals until the end of the re-exposure (this animal also had the lowest baseline before re-exposure [27°C, see Fig 2]). Nevertheless, even though the difference was most marked in the last 10 min, statistical analysis revealed a significant difference between the two groups over the entire 30 min period \[F(1,14)=5.66; p=0.04\]. Finally, a rebound increase in tail temperature was also observed in sham conditioned rats after the re-exposure, similar to that of fear conditioned animals.

As for the other surface areas, no significant fear conditioning effect could be detected for the back \[F(1,14)=1.42; p=0.26\] (Fig 2). Statistical analysis for paws, eyes and head was not possible mainly due to occasional concealment of these regions in the sham conditioned animals. However, the data available suggest a conditioning effect for paws (with a pattern similar to the tail) but not for eye or head (Figs 2 & 3). No noticeable group difference could be observed for the ear either (see Fig 3).

**DISCUSSION**

This study shows for the first time that freezing immobility evoked by conditioned fear in the rat is associated with a marked cooling of extremities (tail and paws), which occurs at the same time as a general warming of the rest of the body, including other cutaneous territories. Skin temperature depends on skin blood flow and sympathetic vasoconstrictor tone in skin arteries (Felder et al., 1954; Hertzman, 1953; Janig & McLachlan, 1992; Blessing & Seaman, 2003; Rand et al., 1965; O’Leary et al., 1985; Hales et al., 1978; Owens et al., 2002). Thus, the result indicate that fear has a strong effect on the sympathetic vasoconstricior outflow to skin arteries, however, the most important finding is that this response is regionalized and limited to extremities.
Changes during re-exposure

Skin temperature

Conditioned fear caused the temperature of the tail to drop down to room temperature. The effect was strong & lasted for the entire duration of the re-exposure. Tail temperature also dropped in the sham conditioned animals but the effect was not as strong, and it only occurred during the first half of the re-exposure. This is entirely consistent with the findings of Blessing & coworkers that even mild alerting stimuli can evoke vasoconstriction in the tail artery of conscious rats (Garcia et al., 2001). It indicates that tail blood flow is very sensitive to the level of arousal and that the stronger the arousal, the stronger and longer the vasoconstriction. The remarkable sensitivity of the tail blood flow also explains the variability of the baseline tail temperature which may have been caused by transient arousal responses to small noises in the experimental room.

Skin temperature also dropped in the paws of both forelimbs and hindlimbs. The effect was as strong as in the tail and showed the same difference between fear and sham conditioned animals as for the tail. In fact, the effect was even more pronounced than in the tail, because baseline paw temperature was higher and less variable. This latter observation suggests that paw blood flow in the rat may be less sensitive to mild alerting stimuli but may respond as well as the tail to stronger arousing stimuli.

There was no decrease in temperature in the other surface areas imaged, including the ear. In fact, a general warming was observed, which was comparable to the increase in body temperature and with no specificity to fear. The back appeared to warm up slower than the eyes and head, most probably because of its thicker layer of fur. These observations indicate that there was no or little skin vasoconstriction in response to arousal (strong or mild) and that the
warming of the skin (which was transmitted to the fur) may have been passive, that is, it was due to the increasing temperature of the blood coming from the warming core.

Thus, only the skin of the tail and paws may vasoconstrict in response to fear and arousal in the rat. Interestingly, a very similar finding was reported in a previous study in the monkey (Baker et al, 1976). In this study, skin temperature was recorded with thermocouple electrodes placed in various skin territories of restrained monkeys exposed to visual and auditory alerting stimuli. When applied, these stimuli evoked marked drops in skin temperature in limb extremities (10-13°C), and in tail, nose and ear (6-7°C) but not in proximal limbs, trunk and most of the head. Thus, except for the changes in the ear, the regional pattern of skin vasoconstriction in the monkey exposed to stressful stimuli is very similar to that seen in the rat exposed to mild or strong (fear) stressful stimuli. This is also in agreement with earlier studies in man which show vasoconstriction in hands, fingers and feet but not forearm, leg or forehead in response to pain, loud noise or mental arithmetic (Grant and Pearson, 1937; Abramson and Feris, 1940; Hertzman and Roth, 1942). This clearly shows that skin vasoconstriction in response to stressful stimuli is not a generalized response but a regionally specific response, contrary to Nalivaiko & Blessing (1999) suggestion.

**Body temperature**

Re-exposure was associated with an increase in body temperature in both groups of animals. This increase may be due in part to the vasoconstriction in tail and paws (less heat loss) and in part to heat generating mechanisms such as increased metabolism in the brain and elsewhere in the body and muscle activity or tension (McGregor et al., 1994; Gordon, 1990; Kiyatkin et al, 2002).

To our surprise, the body temperature response of fear conditioned rats was delayed by 7 min, and although it peaked higher, it remained paradoxically lower than that of the sham conditioned rats for most of the re-exposure. Interestingly, Antoniadis et al (2000), who used intraperitoneally implanted radio-telemetric
probes similar to ours, also found that body temperature was higher in sham than in fear conditioned animals. In contrast, Godsil et al. (2000) who used rectal probes found the exact opposite. In addition, Baker et al (1976) also describe a steady increase in brain temperature in their monkeys exposed to alerting stimuli. These contradictory observations are difficult to reconcile, however, there could be one simple explanation, which is more mechanical than biological. Fear is associated with defecation and ultrasonic vocalizations (not recorded here but see Carrive 2002; Walker & Carrive, 2003) which cause intraperitoneal pressure to increase as abdominal muscles contract. We suspect that this increase in pressure pushed the telemetric probe against the colder abdominal wall, resulting in a cooling of the probe and its thermistor and therefore in a delay of the rise of its temperature at the beginning of the re-exposure. For the same reason, as the probe resumed its deeper position in the peritoneal cavity at the end of the re-exposure, its temperature continued to increase, peaking with the same delay as at the beginning of the re-exposure. Thus, if correct, it may well be that our recordings and those of Antoniadis et al (2000) were shifted because of movement of the probe in the peritoneal cavity of fear conditioned rats (sham conditioned rats did not vocalize nor defecate). The actual increase in body temperature associated with fear might therefore have been higher than that of sham conditioned animals for most of the re-exposure as Godsil et al (2000) study suggests.

Changes after re-exposure

Finally, coordinated changes in body and skin temperature were also observed during the recovery period after re-exposure. These are related to thermoregulatory mechanisms. We observed that the beginning of the decline in body temperature corresponded to a rebound increase in tail temperature 3.3 °C warmer than baseline. The paws did not show such a rebound but instead slowly came back to baseline. The other skin areas, ie, of the head and back, also gradually came back to baseline temperature after the end of the re-exposure. This suggests that the tail played the main role in dissipating the heat
accumulated during the re-exposure by rapidly switching from a vasoconstricted
to a vasodilated state. The importance of the rat’s tail in thermoregulation is well
known (Rand et al., 1965; O'Leary et al., 1985; Hales et al., 1978; Vanhoutte et
al., 2002; Gemmell & Hales, 1977; Gordon, 1990). Our data confirm it, and
suggest that it is possibly the only skin area capable of actively dissipating
naturally generated internal heat in the rat.

Functional significance
The fear response can be seen as a preparatory response to a more active
reaction such as fight or flight. Some components of the response like the
increase in arterial pressure and activation of cardiac sympathetic output prepare
the organism for a quick response, while other components like the freezing
immobility and concomitant activation of cardiac vagal output serve to hold this
active response until the appropriate moment (Walker & Carrive, 2003). The
decrease in skin blood flow in extremities, which like the rise in blood pressure
and heart rate is sympathetically mediated, may also be a component of this
anticipatory response.

A reduction in blood flow to the skin of the tail and paws would contribute to the
redistribution of blood to more important organs such as the brain and muscles,
much like the vasoconstriction that occurs in the viscera. This is clearly
advantageous, however, if it was the main reason for skin vasoconstriction in
response to stress, one may wonder why it is restricted to extremities and not
generalized, even with strong stressful stimuli like conditioned fear. As pointed
out by Blessing (2003), skin vasoconstriction might also be a protective
mechanism to reduce blood loss in case of injury. If this was the case, it would
make sense that skin vasoconstriction should occur in the extremities, because
these areas are the most likely areas to be injured in case of a fight or flight.
Furthermore, extremities are also more likely to lose blood, because they have
arteriovenous anastomoses used for thermoregulation (tail) or greater
vascularisation (paws) than in other parts of the body (Gemmel & Hales, 1977;
Rendel et al., 1998). Other cutaneous areas are less likely to be injured, first
because they are not in contact with the ground or less likely to be caught by a predator and two because they are covered by a protective layer of fur. There may also be species-specific adaptations. For example, the ear of the rat does not appear to vasoconstrict whereas the ear of the rabbit does (Nalivaiko & Blessing, 1999). This may be because the rat’s ear is small, can retract and does not have arteriovenous anastomoses, whereas the rabbit’s ear is large, exposed and rich in arteriovenous anastomoses (Gemmel & Hales, 1977).

Thus, the main reason that skin vasoconstricts in the tail and paws, and in extremities in general, in response to stressful stimuli may well be in anticipation of injury to protect these more exposed areas and reduce a potential blood loss. It also makes sense that the stronger the arousal, the stronger this effect.

ACKNOWLEDGEMENTS

We wish to thank Ms Pip McCahon for the lease of the infrared camera and Dr Barbara Eckersley for her help in the acquisition of the infrared images. This study was supported in part by a grant from the National heart Foundation of Australia. Dr Vianna was supported by a Brazilian fellowship from the CNPq.
REFERENCES


Felder, D., Russ, E., Montgommery, H. & Horwitz, O. (1954). Relationship in the


FIGURE LEGENDS

**Figure 1.** Changes in heart rate, mean arterial pressure, body, back and tail temperature, freezing and activity evoked by re-exposure to the shock box in fear and sham conditioned rats. No shock was given during the test. Back and tail temperatures are represented at the same scale, but body temperature is shown at 2.5 times that scale. There were 6 animals per group but body temperature was actually measured in only 5 animals in each group. Mean ± S.E.M. A repeated measure analysis of variance done over the entire 30 min period of re-exposure revealed a fear effect for mean arterial pressure, tail temperature, freezing and activity (see text for details).

**Figure 2.** Individual data points showing changes in eyes, head, back, paws and tail surface temperatures during the same re-exposures and in the same animals as on Fig 1. Each dot represents one individual temperature measurement extracted from infrared images. The temperature scale is the same for all areas.

**Figure 3.** Infrared digital images of one fear conditioned and one sham conditioned rat obtained before, during and after re-exposure to the shock box. Note the drop in paw and tail temperature in the fear conditioned, but not in the sham conditioned rat during re-exposure. The cold spots over the fur are artifacts due to water drops. All images use the same color coding for temperature.