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# Does exposure to chronic stress influence blood pressure in rats?

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ABSTRACT

The principal aim of this study was to determine whether prolonged chronic footshock stress can evoke sustained changes in blood pressure in rats and to elucidate possible underlying neurochemical mechanisms as mediated by the sympathoadrenal system. Adult male Wistar rats instrumented for telemetric recording of arterial pressure, heart rate and locomotor activity were subjected to six weeks of inescapable unpredictable electrical footshocks (FS+) or were exposed to shock chambers but were not shocked (FS−). Compared to FS− animals, FS+ animals had significantly reduced body weight gain (by 30%), locomotor activity (by 25%) and social interaction time (by 30%) — symptoms commonly induced by chronic stress and depression in humans. These changes were associated with small, but significant increases in systolic blood pressure (by 7%) and pulse pressure (by 11%) in FS+ rats relative to FS− rats. We have also found neurochemical alterations in sympathoadrenal pathways (that lasted for at least one week post-stress) including about 2–3 fold increases in the levels of tyrosine hydroxylase phosphorylation in the sympathetic ganglia and adrenal gland and a 1.8-fold increase in the expression of the Angiotensin II receptor type 1 protein in the adrenal gland of FS+ rats relative to FS− rats. We conclude that uncontrollable and unpredictable footshock stress can lead to elevation in systolic blood pressure when applied for an extended period of time (six weeks) in Wistar rats, and that these changes could be mediated by stress-induced modifications in sympathoadrenal pathways.



## 1. Introduction

Epidemiological studies have demonstrated that individuals exposed to chronically stressful life events frequently exhibit persistent hypertension (Henry and Cassel, 1969; Pickering, 2004; Steenland et al., 2000; Timio et al., 1988). Importantly, studies investigating stress-linked hypertension have observed that elevations in arterial pressure (AP) can persist long after the termination of stress events, and in many instances continue during sleep (Schnall et al., 1992). Together these findings suggest that psychological stressors alter the regulation of AP during stress exposure and that these alterations persist well beyond the duration of stressor.

Thus far the large majority of research that examined the relationship between stress and AP has focused on acute stress. While these studies have been informative from the perspective of understanding how acute challenges provoke rapid physiological alterations, the models used do not replicate the conditions under which stress leads to sustained hypertension in humans. Certainly, there have been several studies that have examined the impact of chronic stress on AP but the results have been difficult to interpret (see Nalivaiko (2011) for recent review). For instance, several studies investigating effects of chronic stress on AP have used the tail-cuff procedure to measure blood pressure in experimental animals. It is now well recognised that this procedure causes substantial pressor and tachycardic responses (Van Acker et al., 2001), as well as elevation in plasma noradrenaline and Angiotensin II (Ang II) concentrations, even following recommended habituation (Grundt et al., 2009). It is quite possible therefore that the changes in AP following tail cuff recordings are attributable to the approach taken to acquire the measurements.

Several studies have, nevertheless, employed the more reliable biotelemetry method or indwelling catheters to measure blood pressure. Reports using this more refined approach have consistently shown that chronic stress does not influence AP (Gelsema et al., 1994; Grippo et al., 2003; Lemaire and Mormède, 1995). Critically, however, these reports have used relatively mild forms of stress. Moreover, the amount of stress experienced by the animals subjected to interventions such as social defeat, social isolation or chronic mild stress is difficult to quantify. As such, it is not entirely clear whether the reported negative results should be interpreted as indicating that chronic stress cannot alter AP in rodents or that the intensity of the stress was simply insufficient to

*Abbreviations:* FS, footshock; AP, arterial pressure; TH, tyrosine hydroxylase; Ang II, Angiotensin II; AT1R, Angiotensin II receptor type 1; Ser, serine residue.

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cause a measurable change. This latter possibility seems considerably more likely as it is widely recognised that principle determinant of a stressful experience is the degree to which it is unpredictable and uncontrollable (Maier, 1984). Having actual or perceived control over a stressful situation powerfully reduces its negative physiological consequences. It is possible therefore that in previous studies in which animals were exposed to relatively mild stressors and also allowed the animal a significant degree of latitude with respect to behavioural modification of the experience may have mitigated the overall impact of the exposure regimen.

Consequently, the primary aim of the current study was to determine whether uncontrollable and unpredictable footshock, an extremely well characterised and easily quantifiable stressor, could evoke a sustained elevation in AP if applied for an extended period of time (six weeks). Importantly, we used constant telemetric monitoring of arterial pressure and heart rate. Additionally, some established stress-linked behavioural changes were monitored using the social interaction and locomotor activity tests. These tests were chosen for confirming that our stress protocol was potent enough to elicit symptoms typically present in chronically stressed or depressed patients — reduced motivation and reduced motor drive (Grippo and Johnson, 2009; Hammen, 2005). We also intended to investigate the impact of chronic footshock stress on activation of the sympathoadrenal system, as chronic activation of this system can lead to increased catecholamine release from the sympathetic nerve terminals and adrenal gland and therefore may contribute to a number of cardiovascular disorders in humans and experimental animals including hypertension (Grassi et al., 2008; Grassi et al., 2010; Lee et al., 1991; Parati and Esler, 2012). Specifically, we assessed the expression and phosphorylation of tyrosine hydroxylase (TH) and the levels of Ang II receptor type 1 (AT1R) in the adrenal gland and in the stellate ganglia — the indices previously associated with chronic stress and/or sympathetic activation (Baruchin et al., 1990; Cierco and Israel, 1994; Dendorfer et al., 2002; Fluharty et al., 1983; Kvetnansky et al., 2004; Ma et al., 2001; Nankova et al., 1996; Powis and Obrien, 1991; Stromberg et al., 1991).

## 2. Materials and methods

### 2.1. Ethical approval

All animal procedures were approved by the Ethics Committee of the University of Newcastle (Australia) and animals were cared for according to the principles of the Australian code of practice for the care and use of animals for scientific purposes (7th edition 2004).

### 2.2. Experimental animals

Experiments were conducted on adult male Wistar rats (between 7 and 10 weeks of age). All animals were housed individually in an animal holding facility, with rat chow and water available *ad libitum*. The room temperature was maintained at 21 °C ± 1 °C and animals were kept on a reversed 12 h:12 h light/dark cycle (lights on at 19.00 h).

### 2.3. Telemetry blood pressure recording

Telemetry probes (TA11PA- C40, Data Sciences International USA) were implanted one week prior to the commencement of the experimental protocol. Surgery was conducted under 2% isoflurane in 100% oxygen anaesthesia as described previously (Beig et al., 2011). The transmitter's catheter was inserted into the left femoral artery *via* a small incision; with the catheter tip advanced into the abdominal aorta. Animals recovered from anaesthesia were returned to their home cage where they were left for one week. Telemetric transmitters were configured and calibrated both before implantation and after explantation. When we compared calibrations made before implantation and after explantation, we did not find any detectable changes in gain. We assessed not only base level, but also sensitivity (by placing probes in a 50 ml syringe and increasing pressure in the syringe, with the syringe output connected to a manometer). Furthermore, the DSI system incorporated the Ambient Pressure Monitor that made automatic correction for changes in the barometric ambient pressure.

The radiotelemetry system employed in this study allowed recording of AP, heart rate and locomotor activity in freely-moving animals. These parameters were continuously acquired 24 h a day 7 days a week during 7 weeks of the experimental protocol (except 40 min footshock sessions). Cardiovascular parameters (heart rate and mean AP) were acquired using the Dataquest ART software (Data Sciences International, USA). The Data Sciences program compressed the data into 10 second averages for systolic blood pressure, diastolic blood pressure and subsequently calculated pulse pressure and mean arterial pressure from these values. Due to the sheer size of the data we then further compressed the raw data into daily averages and weekly averages.

### 2.4. Chronic footshock stress protocol

Footshock procedure was performed by placing animals into a perspex footshock cylinder (22 cm long × 9 cm diameter) located inside an enclosed box. At the commencement of the footshock session the programmable animal shocker (San Diego Instruments, USA) delivered an electrical current (1.5 mA) through the grids to the feet of the animal for 1 s. Each footshock stress session lasted 40 min during which time the animal received six shocks, with a randomly allocated interval between each shock to avoid predictability. Following each session the animals were returned to their home cage. The condition of the animal feet was monitored regularly to ensure that there were no adverse effects of the footshock procedure.

Animals were randomly assigned to two experimental groups: footshock-sham (FS− group; n = 6) and footshock-stress (FS+ group; n = 6). FS+ animals were placed in the footshock apparatus and received footshocks as described above. FS− animals were placed in the footshock apparatus but did not receive footshocks. The experiment lasted for seven weeks in total for each group. During week 0 the animals were left undisturbed in order to obtain a measure of resting baseline parameters. During weeks 1–6, FS+ animals were exposed to the footshocks three times a week (weeks 1–4) or seven times a week (weeks 5–6). During the post-stress week 7, animals were re-exposed to shock chambers (without shocks) for 5 min, with concurrent recording of ultrasound vocalisation. Animals were weighed once a week. Animals were euthanized at the end of week 7 by an anaesthetic overdose, sodium pentobarbital (Lethabarb, 80 mg/kg i.p.) as reported in our previous study (Damanhuri et al., 2012). Stellate ganglia and adrenal glands were collected for biochemical assessment of TH and AT1R expression.

### 2.5. Assessment of animal behaviour

#### 2.5.1. Ultrasonic vocalisation

During week 0 (pre-stress) experimental animals were placed in the footshock apparatus and remained within these boxes for 1 min without being shocked. During this time 22-kHz ultrasonic vocalisations were recorded using a MiniBat ultrasound microphone (Ultra Sound Advice, UK) and acquired using lab chart software (ADI instruments, Australia). During week 7 (post-stress) all experimental animals were once again placed into the same footshock apparatus that they were exposed to throughout the experimental protocol. Animals remained within these boxes for 1 min without being shocked. During this time 22-kHz ultrasonic vocalisations were recorded in order to detect alterations in animal vocalisation following chronic stress.

#### 2.5.2. Social interaction test

The test was performed during week 7. Social interaction was tested in a square arena 50 × 50 × 40 cm all painted in black under low light (Overstreet and Griebel, 2004). Rats were paired up with a naive, age-