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Expression of cardiovascular and behavioural components of conditioned fear to context in T4 spinally transected rats

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Abstract

A spinal cord transection at the fourth thoracic level (T4) results in paraplegia. It also removes supraspinal control of sympathetic outflow to most viscera and their blood vessels but spares the heart. We studied the effects of such a transection on the expression of the conditioned fear response to context, which includes freezing, 22 kHz ultrasonic vocalisations, a marked pressor response and a slowly rising tachycardia. Rats implanted with radiotelemetric probes were fear conditioned, tested, then transected at T4 and finally re-tested 4 weeks after transection. Baseline blood pressure in transected animals was the same as in intact animals but baseline heart rate was 127 bpm higher. There were clear signs of fear in the transected animals: although freezing occurred in the upper part of the body only, there was a 3 fold increase in the number of ultrasonic vocalisations, most probably due to paralysis of abdominal muscles that made expirations shorter and therefore more frequent. The pressor response of fear was initially the same as in intact animals but controls revealed that this was due to handling during transfer to the aversive context. The rest of the pressor response was markedly reduced (70%) confirming that it depends in large part on a sympathetically mediated increase in vascular resistance in the lower part of the body. The cardiac response was characterized by an initial bradycardia followed by a marked tachycardia, which is consistent with a baroreceptor-mediated reflex response to the altered pressor changes. Finally, none of these changes was observed when the same experiment was repeated in sham transected animals. Thus, the pressor response of fear is in large part mediated by the thoracic cord below T4 and the baroreflex is not inhibited but maintained during conditioned fear.

Keywords: Conditioned fear; Radio-telemetry; Blood pressure; Heart rate; Ultrasonic vocalisation; Freezing behaviour; Autonomic dysreflexia; Baroreceptor reflex

1. Introduction

Conditioned fear to context is a conditioned emotional response which can be obtained, for example, by re-exposing an animal to the same box in which it has previously received electric foot shocks (Fendt and Fanselow, 1999; Maren, 2001). The fear of receiving more shocks in the box is what generates this emotional response. The fear response is characterised by a freezing immobility, ultrasonic vocalisations, a marked rise in blood pressure and a slow increase in heart rate as well as other signs of autonomic activation such as defecation and urination (Carrive, 2002). Previous work from our laboratory has shown that the pressor response of fear is sympathetically mediated because it can be abolished by pharmacological blockade of autonomic ganglia or antagonism of α-adrenergic receptors (Carrive, 2002). Thus, the pressor response must be mediated by descending projections to the thoracic cord where the preganglionic sympathetic neurons (SPNs) that control sympathetic outflow are located (between spinal segments T1 and L3).

A spinal transection at thoracic level T4 will result in loss of somatosensory and visceral sensations in the lower half of the body as well as loss of voluntary control of the hindlimbs or paraplegia. It will also remove supraspinal control of sympathetic outflow to the lower part of the body

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including abdominal viscera and their vasculature, but spare that of the upper body, including the heart (Strack et al., 1988). The question is: will T4 transected rats still show signs of fear, and if they do, how different will their cardiovascular response be if most of the sympathetic vasomotor outflow is lost but control of the heart is spared? The aim of this experiment is in part to verify the findings of the pharmacological studies described above and in part to better define the role of the spinal cord below T4 in shaping the fear response. To answer these questions we tested conditioned fear to context in chronic paraplegic rats that had been conditioned before a spinal transection at T4. To the best of our knowledge, this has never been reported before.

2. Materials and methods

The subjects were 20 experimentally naïve male Wistar rats (400–450 g) obtained from the colony of pathogen-free rats maintained by the University of New South Wales. They were housed in individual plastic home boxes ($65 \times 40 \times 22$ cm) during the whole experiment. All procedures 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. The rats were anaesthetized with a mixture of ketamine and xylazine (100 and 50 mg/kg, i.p., respectively) and telemetric probes (PA-C40, Data Sciences International) were implanted in the peritoneal cavity as previously described (Carrive, 2000). Briefly, a midline incision was made in the abdomen and the descending aorta was exposed at the level of the iliac bifurcation. The artery was punctured at this level and the tip of the catheter (1 cm) was inserted. The puncture was then glued with the catheter in place and the probe was stitched to the abdominal wall while closing the midline incision. The animals were given antibiotics and allowed to recover for 1 week before conditioning started.

Fear conditioning was also conducted as previously described (Carrive, 2002). It was carried out in foot shock chambers (23 cm × 21 cm × 20 cm) equipped with a grid floor wired to an electric shock generator. The chambers were cleaned with 0.05% of acetic acid solution prior to their use. Preconditioning consisted of two 5-min-long pre-exposures to the foot shock chamber on consecutive days without shock. Conditioning shock sessions were conducted on separate days over a period of 7 days. There were three shock sessions. Each consisted of a 40-min-long exposure to the foot shock chamber during which four electric foot shocks (1 mA, 1 s) were delivered at approximately 5, 15, 25, 35 min. Contextual fear was then tested by re-exposure for 30 min to the aversive context with no shock delivery. The response was recorded as described below. A fourth and last shock session was given prior to the spinal transection.

For spinal transection, the rats were re-anaesthetized with ketamine and xylazine as above. The spinal cords were then exposed by T3 laminectomies and transected at the T4 level (Leman et al., 2000; Leman and Sequeira, 2002). To ensure a complete transection, the two stumps of the cord were separated and a small piece of sterilised gelfoam was introduced in between. The muscle, fascia and skin were then sutured in layers. Intensive post-operative care was given during the first week that followed the surgery. The animals were kept in a warm environment and temperature was monitored for the first week or until stable. Antibiotic and saline were administered twice per day for 3 days and manual compression for bladder voidance was applied until automatic voiding occurred (approximately 2 weeks after transection).

Only 14 rats were transected. In the remaining 6 rats, the spinal cord was exposed and the dural sac pierced but the cords were not transected. These animals were used as sham transected controls.

The rats were kept for up to 9 weeks after spinal surgery and during this period they did not receive any more electric foot shock. Contextual fear was finally tested by re-exposure for 30 min to the aversive context as described above. Nine of the 14 transected rats were tested 4 weeks after the transection. The other 5 rats were tested 9 weeks after the transection. Three of the 6 sham transected controls were tested 4 weeks after the laminectomy and the other 3 were tested 5 weeks after the laminectomy. In addition, 4 of the transected rats were used as handling controls during the fourth week after transection. These animals were taken from their home box and handled for about 5 s to mimic the effect of transfer from the home box to the shock box. The animals were then returned to their home box. Handling was also tested in 4 intact rats for comparison.

Five parameters were recorded when testing conditioned fear: heart rate (HR), mean arterial pressure (MAP), activity, freezing and 22 kHz ultrasonic vocalisation. HR, MAP (both sampled for 3 s every 30 s) and activity for body movements were derived from the telemetric probe signal and acquired automatically by the A.R.T. system (Data Sciences International, Inc.) before, during and after the re-exposure. Freezing and the number of ultrasonic vocalisations were recorded manually by an experimenter located in the experimental room. Freezing (sampled every 2 s) was defined as a total absence of body or head movement except that associated with breathing (Antoniadis and McDonald, 1999). 22 kHz ultrasonic vocalisation was detected by an ultrasonic bat detector (Mini-3, Ultrasound device). Finally, all parameters were averaged (HR, MAP, Activity) or cumulated (Freezing, Ultrasonic vocalisations) over 1-min period.

Data were analyzed with StatView 5.0 using one-way repeated measures ANOVA. The independent factor was spinal transection and the repeated measure was time. Statistical significance was set at $P = 0.05$.