Effects of midazolam and naloxone in rats tested for sensitivity/reactivity to formalin pain in a familiar, novel or aversively conditioned environment

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Abstract. Rats tested for sensitivity/reactivity to formalin-induced pain in either an aversively conditioned or a novel environment displayed immediate but transient hypoalgesic responses that were insensitive to either a benzodiazepine (midazolam) or an opioid antagonist (naloxone). Exposure to the aversively conditioned, but not to the novel environment also provoked a more enduring hypoalgesic response that was abolished by either midazolam or naloxone. The results were taken to mean that fear is sufficient but not necessary for the production of hypoalgesic responses to environmental stimuli.

Key words: Formalin test - Hypoalgesia - Fear - Novelty - Conditioning - Midazolam - Naloxone

It has been well established that hypoalgesic and fear responses can co-occur in the presence of environmental stimuli which have become dangerous as a result of their association with a noxious event. For example, rats failed to lick or lift a formalin-injected paw in an environment where they had previously received footshock but did lick that paw in a safe one. In contrast, rats displayed a freezing response in the dangerous environment but were active in the safe one (Fanselow and Baackes 1982). Similarly, intruder rats defeated in an agonistic encounter did not lick a formalin-injected paw while freezing in the presence of colony odours associated with the resident male rat (Williams et al. 1990). Finally, rats tested on the heated floor of a hot-plate apparatus took longer to initiate paw-licking when they had been previously exposed to the 54°C floor of that apparatus than rats pre-exposed to 52°C or 50°C floors. Moreover, rats that had been exposed to the 54°C floor also displayed fear, taking longer to step down from a platform onto the 23°C floor of the hot-plate apparatus than rats pre-exposed to 52°C or 50°C floors (Westbrook et al. 1991).

The co-occurrence of hypoalgesic and fear responses in the presence of environmental stimuli associated with a noxious event is consistent with the view that hypoalgesia is a response to fear (Bolles and Fanselow 1980; Chance 1980). Fanselow (1986), for example, has argued that innate and learned danger signals activate a fear motivational system which functions to select and release species-typical behaviours tailored to cope with environmental sources of danger. However, regardless of the particular defensive behaviour selected, activation of the fear system is assumed to inhibit a pain intensity detector, thereby provoking hypoalgesia. Fanselow and Helmstetter (1988) provided support for this argument by showing that the freezing and hypoalgesic responses to shock-related cues were reduced in rats tested under the influence of a benzodiazepine. The sensitivity of conditionally elicited hypoalgesic responses to a benzodiazepine has been confirmed by other investigators. For example, Maier (1990) reported that the hypoalgesic response to shock-related cues was reduced or eliminated by diazepam. Similarly, Harris et al. (1993) found that a low dose of midazolam attenuated the conditioned hypoalgesic response resulting from pre-exposure to the 54°C floor of the hot-plate apparatus. Although thus documenting a role for fear in the production of a hypoalgesic response to learned danger signals, benzodiazepine-insensitive hypoalgesic responses were also observed in the Maier (1990) and Harris et al. (1993) studies. Specifically, the hypoalgesic responses elicited by shock (Maier 1990) or by exposure to a novel hot-plate apparatus (Harris et al. 1993) were unaffected by benzodiazepines, indicating that these forms of environmental stimulation activated anti-pain mechanisms independently of fear.

The present experiments tested rats for sensitivity/reactivity to a formalin-injected paw (Dubuisson and Dennis 1977) on the non-heated floor of a hot-plate apparatus to provide a further examination of the hypoalgesic responses provoked by exposure to novel or aversively conditioned environmental stimuli. Experiment 1 was designed to confirm the presence of hypoalgesic responses in rats exposed to a novel test environment (Abbott et al. 1986) and in rats pre-exposed to the 54°C floor of a
hot-plate apparatus (Westbrook et al. 1991). Experiment 2 examined the effects of extinction upon the aversively conditioned hypoalgesic response resulting from pre-exposure to the 54 °C floor. The remaining experiments investigated the effects of 1) a benzodiazepine (midazolam) and 2) an opioid antagonist (naloxone) to determine the involvement of fear and of opioid antipain mechanisms in the production of the hypoalgesic responses to novel or aversively conditioned test environments. Thus, experiments 3, 4 and 5 tested rats under the influence of midazolam in a familiar, novel or aversively conditioned apparatus, respectively. In experiment 6, rats were injected with both midazolam and a benzodiazepine antagonist (flumazenil) to show that the effects of midazolam on hypoalgesic responses were due to the drug's action at the benzodiazepine receptor. The two final experiments examined the effects of naloxone (experiment 7) and of a combination of naloxone and midazolam (experiment 8) upon the hypoalgesic responses to a novel and aversively conditioned apparatus.

Materials and methods

Subjects

Experimentally naive, male Wistar rats, weighing between 310 and 440 g, were obtained from the colony of Specific Pathogen Free rats maintained by the University of New South Wales. They were housed in plastic boxes (65 × 40 × 22 cm) with eight rats per box and with continuous access to food and water. The boxes were kept in a colony room under natural lighting. The experiments were conducted between 0900 and 1400 hours.

Apparatus

The hot-plate apparatus consisted of a Plexiglas cylinder (24 × 48 cm, diameter × height) with a copper floor fixed 12 cm above the base of the cylinder. The portion of the cylinder below the copper floor was perforated with holes (diameter 3 cm) to permit circulation of water beneath the copper floor. The cylinder stood in a water bath that could be maintained at a particular temperature (∼0.5 °C) by a Haake D1 Open Bath Circulator. Plastic buckets (26 × 26 cm, diameter × height) with air holes drilled in the lid and side served as chambers where the rats were kept in isolation when brought back to the laboratory.

Drugs

All drugs were administered in a volume of 1.0 ml/kg. Midazolam (Hypnovel; Roche) was diluted with isotonic (0.9% w/v) saline, and given as intraperitoneal (IP) injection 20 min before testing. Flumazenil (Roche Products) was suspended in a vehicle solution of distilled water to which was added two drops of Tween 80 per 10 ml, and injected IP 30 min before test. Naloxone hydrochloride (Sigma) was dissolved in isotonic saline and injected subcutaneously (SC) into the dorsal region of the neck at a dose of 5.0 mg/kg 30 min before test. Control injections of saline were given at the same time as the relevant drug injection, and were administered IP as a control for midazolam or flumazenil, and SC as a control for naloxone.

Procedure

Familiarisation. On days 1–4, rats to be tested in a familiar or aversively conditioned apparatus were placed in plastic buckets for 30 min and then exposed to the 23 °C floor of the hot-plate apparatus for 60 s. On these days, rats to be tested in a novel apparatus were given equivalent handling in the colony room.

Conditioning. On day 5, rats to be tested in an aversive apparatus were placed in the plastic buckets for 30 min and then exposed for 30 s to the 54 °C floor of the hot-plate apparatus; rats to be tested in the familiar apparatus were placed in the plastic buckets and given a 30 s exposure to the 23 °C floor of the apparatus; rats to be tested in a novel apparatus were handled in the colony room.

Testing. In each experiment, rats were tested on day 6 except in experiment 2 when rats were tested on day 9. Thirty minutes before testing, each rat was anaesthetized for approximately 3 min with 5% halothane (Fluothane, ICI) delivered in 750 mm Hg nitrous oxide and 300 mm Hg oxygen. This was done so as to permit precise injection of formalin, and at the request of the University's ethics committee. While still unconscious, rats were injected with 0.05 ml of a 5% formalin solution (diluted with distilled water) into the plantar surface of the right hind paw. Rats were then placed in the plastic buckets for 30 min, to allow full recovery and elimination of the anaesthetic. Rats were then placed onto the 23 °C floor of the hot-plate apparatus for 5 min, while an observer used push-buttons connected to a computer to record the occurrence of responses to the injected paw. A response was defined as lifting (holding the injected paw off the ground while bearing weight on its other paws) or attending (biting or licking the injected paw). The mean time spent responding (lifting and attending) per minute was calculated for the 5 min of test. The observer was unaware of the groups to which the rats had been assigned.

Experiment 1: effects of testing in a familiar, novel or aversively conditioned apparatus on formalin responding

Three groups of rats were used (n = 10). One group had been familiarised and then exposed to the 54 °C floor of the hot-plate apparatus, one group had been familiarised with the apparatus but not exposed to the heated floor, while the third group had never been exposed to the apparatus prior to test.

Experiment 2: extinction of conditioned hypoalgesia

Four groups of rats (n = 10) were familiarised with the apparatus before being exposed either to the 54 °C or 23 °C floor of the apparatus on day 5. Rats conditioned by exposure to the 54 °C floor then received extinction training (Group Conditioned/Extinguished) or equivalent handling in the colony room (Group Conditioned/Not Extinguished). Extinction training consisted of placing rats in the plastic buckets for 30 min before exposing them to the 23 °C floor of the hot-plate apparatus for 10 min on each of 3 consecutive days. Half the rats exposed to the 23 °C floor on day 5 were given corresponding “extinction” training (Group Familiarised/“Extinguished”) while the other half were handled in the colony room (Group Familiarised/“Not Extinguished”).

Experiments 3, 4 and 5: effects of midazolam on formalin responding in a familiar, novel or aversively conditioned apparatus

In each experiment, three groups of rats (n = 8) were injected with either midazolam (1.0 mg/kg or 2.5 mg/kg) or saline, 20 min before test, i.e., 10 min after formalin injection. Rats in experiment 3 had been familiarised with the apparatus but not conditioned; rats in experiment 4 had never been exposed to the apparatus prior to test; and rats in experiment 5 had been familiarised with the apparatus and then conditioned by exposure to the 54 °C floor.