Physiology & Behavior 95 (2008) 252–257

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Caffeine dependence in rats: Effects of exposure duration and concentration

Rachel N. Dingle *, Sarah E. Dreumont-Boudreau, Vincent M. LoLordo

Dalhousie University, Halifax, Nova Scotia, Canada B3H 2J1

ARTICLE INFO

Article history:
Received 25 March 2008
Received in revised form 12 May 2008
Accepted 10 June 2008

Keywords:
Caffeine
Rats
Conditioned avoidance
Dependence
Withdrawal
Dose
Associative learning

ABSTRACT

Groups of rats were chronically exposed to a 1.0-g/L caffeine solution for 5, 10, 15 or 20 days. Upon removal of caffeine, rats were given brief exposure to a novel flavour CS (withdrawal CS) followed by 12 days of plain water and then brief exposure to a second flavour CS (neutral CS). Only rats exposed to 20 days of caffeine strongly preferred the neutral CS to the withdrawal CS in a 2-bottle test. In Experiment 2, groups of rats were chronically exposed to caffeine at one of four concentrations (1.0, 0.5, 0.25, or 0.125 g/L) for 21 days, after which withdrawal and neutral CSs were established. Only rats that drank the highest caffeine concentration, 1.0 g/L, preferred the neutral CS to the withdrawal CS. This suggests that long exposure to a strong caffeine solution is required in order to induce dependence in rats such that a CS associated with the withdrawal of caffeine becomes avoided.

Caffeine dependence in humans is now well-established [for a review, see [1–3]]. However, unlike humans, other animals have not been found to readily self-administer caffeine. Perhaps because of this hurdle, animal models of caffeine dependence, which would allow for more direct investigation into the systems involved, are less common in the literature than one might expect from the ubiquitous consumption of caffeine by humans.

Physiological withdrawal from caffeine in the rat has been inferred from several changes in behaviour evident upon cessation of a chronic caffeine regimen. In a number of experiments by Holtzman and colleagues [4,5] rats were allowed to drink caffeine in 10-minute sessions every 6 h for 5 or 11 weeks. Upon termination of this long-term regimen, rats exhibited significantly less locomotor activity than baseline. This reduction in activity was found to last 48 h in rats given 5 weeks of caffeine, and up to 4 days in rats given 11 weeks. A subsequent study [6] similarly found that a decrease in the reinforcement threshold for intra-cranial self-stimulation followed cessation of chronic caffeine, opposite to an initial increase in threshold with caffeine onset. It was argued in all of these studies that the pattern of results, a decrease in locomotor activity or ICSS threshold upon caffeine removal opposite to the increase in both with caffeine onset, was consistent with drug withdrawal. More recently, withdrawal from chronic caffeine injections has also been found to elevate anxiety in the rat as measured by reduced entries to, and time spent in, the open arms of an elevated plus maze [7].

Conditioned taste aversion (CTA) is a well-characterized phenomenon initially investigated by Garcia and colleagues [8–10], who found that pairing a novel taste with illness resulted in rats avoiding that taste, even when exposure to the taste and the onset of sickness were separated by a much longer interval than normally used in learning paradigms [for a review, see [11–13]]. Parker, Failor and Weidman [14] successfully used CTA as a behavioural measure with which to investigate morphine withdrawal in rats. After receiving either morphine or saline injections for 25 days, rats were conditioned with access to sucrose-octa-acetate (SOA) solution paired with morphine injections and access to water paired with saline injections. In a two-bottle choice test, morphine addicted rats showed an increased preference for SOA over water, while control rats that had received 25 days of saline showed avoidance of SOA as compared to water. Furthermore, a second batch of rats similarly addicted to morphine showed avoidance of a novel saccharin solution given to them with the cessation of morphine injections. It was inferred from these results that while the physiological effects of morphine are initially aversive, the dependence caused by chronic exposure results in aversive conditioning to flavours associated with the drug's withdrawal and appetitive conditioning to flavours associated with its return. Subsequently, CTA has been used to demonstrate withdrawal in a range of drugs including cocaine, alcohol, and nicotine [15–17].

In 1977, Vitiello and Woods [18] found that rats, after 12 days of receiving caffeine injections immediately after drinking water, learned to reduce consumption of a novel saccharin solution paired with a saline injection (instead of caffeine) as compared to both rats that received another caffeine injection and control rats that received

* Corresponding author. Dalhousie University, Psychology Department, 1355 Oxford Street, Halifax, Nova Scotia, Canada B3H 4J1. Tel.: +1 902 494 3441; fax: +1 902 494 6585.
E-mail address: rdingle@dal.ca (R.N. Dingle).

0031-9384/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2008.06.007

saline in both training and testing. Vitiello and Woods argued that the saccharin solution signalling the absence of caffeine in the chronically exposed rats would only become avoided if this absence was associated with aversive or unpleasant sensations such as are present during drug withdrawal.

Recently, Dreumont-Boudreau, Dingle, Alcolado and LoLordo [19] developed a conditioned taste avoidance (CTA) paradigm for assessing caffeine dependence in rats resulting from chronic oral exposure. Rats were given caffeine-tainted water as their only source of fluid for 21 days. Rats then received a novel flavour after which caffeine was returned to their cages. This flavour was termed the maintenance CS. Two days after the end of maintenance CS training, caffeine was removed and rats received a second novel flavour (withdrawal CS) followed by plain water. Six days after the removal of caffeine, rats were given a third flavour as a neutral CS. In a series of two-bottle tests, rats consumed equal amounts of the maintenance and neutral CSs and showed a significant avoidance of the withdrawal CS relative to the other two. Dreumont-Boudreau et al. concluded that, as in the injection study by Vitiello and Woods [18], rats learned to avoid the withdrawal CS because of associations learned between that flavour and negative caffeine withdrawal symptoms.

In the present experiments, we sought to replicate the effect found by Dreumont-Boudreau et al. [19] using a simplified CTA paradigm involving only 2 flavours (withdrawal and neutral). Furthermore, we wished to define the parametres required for the establishment of oral caffeine dependence leading to withdrawal upon caffeine removal. In two experiments, the duration of caffeine exposure and the concentration of caffeine were varied before CTA training.

1. Experiment 1

This experiment was set up to investigate how different durations of caffeine experience affect the development of caffeine dependence in rats. Dependence was inferred from the occurrence of withdrawal as measured by conditioned avoidance of a flavour paired with caffeine removal (withdrawal CS) in comparison to a second, neutral flavour (neutral CS). As discussed earlier, Dreumont-Boudreau et al. [19] found evidence of withdrawal after 21 days of chronic caffeine. Conversely, Finn and Holtzman [5], measuring locomotor activity, found tolerance to, but no effects of withdrawal from, 4 days of chronic caffeine as measured by locomotor activity. The latter finding has been replicated in our lab using CTA (unpublished data), but without the inclusion of a 21-day group, such that any comparisons made between the 4-day and 21-day groups were between experiments. In the present experiment, groups of rats were exposed to chronic caffeine for 5, 10, 15 or 20 days before flavour conditioning.

1.1. Methods

1.1.1. Subjects

Forty adult male Sprague–Dawley rats (Charles River Canada, Quebec) were used. Their mean weight was 355 g at the beginning of the experiment. Rats were housed separately in shoebox cages in a room maintained at 20–22 °C with a 12:12 light–dark cycle (lights on at 08:00). Food was available *ad libitum* throughout the experiment. All housing and procedures in both experiments were approved by the University Committee on Laboratory Animals; protocol # 06-025.

1.1.2. Apparatus

All training and testing took place in home cages, which were standard shoebox cages made of clear plastic with wire tops. Cages measured approximately 45×25× 20 cm and each contained one 12× 7-cm hiding tube made of black PVC and approximately 2 cm of wood shavings. Solutions were given in 250-mL glass bottles with stainless steel sipper tubes that extended through the wire tops of the cages.

During chronic caffeine exposure, rats had unrestricted access to water mixed with 1.0 g/L caffeine anhydrous powder (Sigma, Canada). The conditional flavour stimuli used were 2.5 g/L grape and cherry Kool-Aid mixed with tap water and sweetened with 1.0 g/L saccharin sodium salt hydrate (Sigma, Canada) to promote drinking. These two flavours have previously been found to be discriminable but equi-preferred [20].

1.1.3. Procedure

Throughout the experiment, caffeine consumption was measured every 24 h between 16:30 and 17:00, except during training. Bottles were weighed (1 g was taken to equal 1 mL) and were replenished as needed. Rats were weighed every second day. Before beginning the experiment, 24-hour baseline water consumption was measured.

During the 6 days prior to conditioning, all rats were habituated to a plain saccharin solution (1.0 g/L) in order to alleviate neophobia and promote drinking of the saccharin Kool-Aid flavour stimuli during training, as well as to familiarize the animals with the training schedule. Each rat received saccharin as its only source of fluid for 2 full days, followed by 4 days of plain water that was removed during a 30-minute saccharin training period between 16:30 and 17:00. Rats were then divided into four groups equated for total saccharin consumption. All rats received water for 24 h after saccharin training and before beginning their chronic caffeine regimen of 5, 10, 15 or 20 days.

For each group, immediately after removal of caffeine, a bottle containing 50 mL of sweetened grape or cherry Kool-Aid (withdrawal CS) was placed on each rat's cage for 30 min (16:30–17:00), after which plain water was returned to all cages. This process was repeated twice more such that rats received a total of three training sessions, at 0, 24 and 48 h after caffeine removal. Bottles were weighed after each session and consumption was recorded in mL. Kool-Aid flavours were counterbalanced within each group to control for any non-associative preference effects.

After withdrawal training, rats were allowed 12 days of plain water to ensure that there were no lingering effects of withdrawal. As was noted earlier, withdrawal was seen at up to four days after removal of caffeine by Holtzman and colleagues [4,5]. At 16:30 on the following day, water bottles were removed and the appropriate second Kool-Aid flavour (neutral CS) was placed on each cage for 30 min. Training proceeded identically to withdrawal training, but for two days rather than three because of an error in protocol.

For the two days following training, all rats received two 24-hour two-bottle tests in their home cage. During the first test, only plain water was available in order to habituate the rats to the availability of two bottles. In the second test, one bottle contained sweetened cherry Kool-Aid and the other grape; the side upon which each rat received its withdrawal CS flavour was counterbalanced.

1.2. Results

The average weight of the rats did not differ across groups throughout the experiment, increasing steadily from 355 to 507 g at the end of 20 days. Mean baseline water consumption at the start of the experiment was 50.0 mL over 24 h. During 24-hour saccharin habituation, mean fluid consumption increased to 95.1 mL. Thirty-minute training consumption of saccharin increased from a mean of 2.7 to 5.5 mL over the four days. During the chronic caffeine phase, mean daily fluid intake did not differ significantly across groups; the 5-, 10-, 15-, and 20-day groups drank 44.7, 45.4, 39.8, and 42.8 mL respectively.

1.2.1. Training

Rats in the 10-, 15-, and 20-day groups drank 4.0, 4.0 and 5.3 mL of the withdrawal CS in three days, about twice as much as what they drank of the neutral CS (2.0, 2.2, 1.9 mL respectively) in two days. Rats