A cognitive deficit induced in rats by chronic intermittent cold stress is reversed by chronic antidepressant treatment

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Abstract
We have previously reported that 14-d chronic intermittent cold (CIC) stress induced a cognitive deficit in reversal learning on the rat attentional set-shifting test. This effect may be related to dysregulation of 5-HT function in orbitofrontal cortex, as a model of cognitive dysfunction in depression. To test the ability of chronic antidepressant drug treatment to reverse the cognitive deficit induced by CIC, it was first necessary to assess the temporal characteristics of the CIC-induced cognitive deficit. Thus, in the first experiment, we assessed the duration of the cognitive deficit following 2-wk CIC stress. Replicating previous experiments, CIC induced a reversal learning deficit tested 3 d after the last cold exposure. However, cognitive performance of CIC-stressed rats was no different from unstressed controls when tested 7, 14 or 21 d after termination of the stress treatment. We next compared behaviour 3 d after 2-wk CIC to that seen 3 d after 5-wk CIC, and found similar deficits in reversal learning. Thus, in the final experiment, antidepressant drug treatment was initiated after 2-wk CIC stress, and was maintained for 3 wk, concurrent with the continuation of CIC stress. Both chronic and acute treatment with the selective serotonin reuptake inhibitor, citalopram, but not the norepinephrine reuptake blocker, desipramine, reversed the cognitive deficit induced by CIC stress. Thus, this stress-induced cognitive deficit may be a useful model for cognitive deficits related to prefrontal cortical hypoactivity in depression, and for investigating neurobiological mechanisms underlying the beneficial effects of chronic antidepressant drug treatment.

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Introduction
Chronic stress is a risk factor for depression and dysregulation of brain monoaminergic neurotransmitters (Anisman & Zacharko, 1986). Monoamines play a role in state-related modulation of executive function, and disturbances of executive function have been implicated in depression (Beck, 1976; Beck et al. 1987). Deficits in executive function, such as impaired cognitive set-shifting, behavioural inflexibility and perseveration (see Fossati et al. 1999), are consistent with the perseverative negative ruminations and emotional biases present in depression (Murphy et al. 2003, 1999), and are associated with prefrontal cortical hypoactivity (Rogers et al. 2004; Sheline, 2003), which resolves with successful antidepressant treatment (Goldapple et al. 2004; Kennedy et al. 2001; Prasko et al. 2004).

In previous studies, we have used an attentional set-shifting test (AST) for rats (Birrell & Brown, 2000), to address modulatory effects of monoamines on cognitive function in prefrontal cortex, and possible mechanisms underlying chronic stress-induced cognitive dysfunction. In the AST, adapted from tests of cognitive flexibility in non-human primates (Roberts et al. 1992), and humans (Beats et al. 1996), rats learn a series of contingencies in which cues within one of two stimulus dimensions (odour or texture) signal the location of a food reward. Once they master the contingency on each successive task, the rules are
changed, and they must suppress the previously successful strategy to learn the new rule and proceed through the next task. The manner in which the rules are changed allows an assessment of different forms of cognitive flexibility, each dependent to varying degrees on the integrity of different subregions of prefrontal cortex (see Birrell & Brown, 2000; Bondi et al. 2008; Lapiz & Morilak, 2006; Lapiz et al. 2007; Lapiz-Bluhm et al. 2009; McAlonan & Brown, 2003). Lesions of medial prefrontal cortex (mPFC) caused selective impairment of extra-dimensional (ED) cognitive set-shifting (Birrell & Brown, 2000), whereas lesions of orbitofrontal cortex (OFC) resulted in selective impairment of reversal learning (McAlonan & Brown, 2003). Previous studies also indicate that these cognitive processes are modulated by monoaminergic neurotransmission. Increasing noradrenergic transmission in mPFC facilitated ED set-shifting (Lapiz & Morilak, 2006; Lapiz et al. 2007), and serotonergic transmission in OFC selectively facilitated reversal learning (Clarke et al. 2004, 2005, 2007).

We have used the AST previously to study chronic stress-induced changes in cognitive flexibility relevant to depression. Chronic unpredictable stress (CUS), a psychogenic stressor, impaired ED set-shifting and reversal learning, and these impairments were prevented by concurrent treatment with desipramine (Des), a selective norepinephrine (NE) reuptake blocker (Bondi et al. 2008). By contrast, chronic intermittent cold (CIC) stress, a metabolic stressor, induced a reproducible and selective impairment in reversal learning that was mimicked by central serotonin (5-HT) depletion (Lapiz-Bluhm et al. 2009). Extracellular levels of 5-HT, measured by microdialysis in the OFC during testing, were reduced in CIC-stressed rats, and the CIC stress-induced reversal learning deficit was attenuated by elevating serotonergic neurotransmission with an acute administration of citalopram (Cit), a selective serotonin reuptake inhibitor (SSRI) (Lapiz-Bluhm et al. 2009). The effect of chronic SSRI treatment has not yet been tested.

In these previous studies, reuptake blockers were administered either acutely, immediately prior to testing, or chronically by osmotic minipump using a preventive strategy, in which drug treatment was initiated prior to the beginning of stress, and was maintained during the course of chronic stress treatment until testing. However, it would be more relevant to the human clinical situation, and would allow a more informative investigation of adaptive mechanisms underlying chronic antidepressant drug effects, if treatment could be initiated after the targeted behavioural effect of stress had been established, in order to test its ability to reverse rather than prevent the stress-induced cognitive deficit. Towards this end, the purpose of the first experiment in this paper was to characterize the duration of the reversal learning deficit induced after 2-wk CIC stress. This experiment replicated the selective deficit in reversal learning shown previously when tested 3 d, but not 7, 14 or 21 d after the final cold stress exposure, indicating that the duration of effect was insufficient for testing the efficacy of 3-wk antidepressant drug treatment initiated after the termination of stress. Thus, in the second experiment, an alternate approach was tested, extending CIC stress treatment to 5 wk, which would allow a 3-wk drug treatment to be initiated after the cognitive deficit had been established at 2 wk. In this experiment, a selective reversal learning deficit was seen after 5-wk CIC stress, similar to that after 2 wk. Thus, the 5-wk CIC treatment protocol was used in the third experiment to test the ability of chronic treatment with a SSRI, Cit, or a selective NE reuptake inhibitor, Des, initiated after 2-wk CIC stress and maintained while stress treatment was continued for an additional 3 wk, to reverse the cognitive deficit induced by CIC stress. Portions of this work have been presented in abstract form (Lapiz-Bluhm et al. 2008).

Methods

Animals

A total of 221 adult male Sprague–Dawley rats, weighing 220–240 g on arrival, were initially group-housed (3 per cage) in 25 x 45 x 15 cm plastic cages, on a 12-h light/dark cycle (lights on 07:00 hours), with food and water available ad libitum. After acclimatizing for at least 7 d, rats were housed individually prior to any experimental manipulation. Experiments were conducted during the light phase of the cycle, between 09:00 and 17:00 hours. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, and were consistent with NIH guidelines for the care and use of laboratory animals. All efforts were made to minimize pain, discomfort, suffering, and the number of rats used.

CIC stress

The procedure for CIC stress exposure was as described previously (Lapiz-Bluhm et al. 2009; Ma & Morilak, 2005). Rats were randomly assigned to control or CIC stress conditions. Rats in the CIC stress group were transported in their home cages, with food, water and bedding, into a cold room (4 °C) for
6 h, then returned to the housing room, every day for 14 consecutive days (expts 1 and 2), or for 5 wk (expts 2 and 3). Control rats remained undisturbed in the housing facility during this time. One week before testing, rats were restricted to 14 g food per day, with water freely available. After the CIC treatment period, rats were taken through the 3-d AST protocol.

AST

The AST was conducted as described previously (Lapiz & Morilak, 2006), in a rectangular wooden arena (length × width × height: 71 × 40 × 20 cm) painted white on all surfaces. A removable divider separated one-third of the arena, forming a start box which also served as a holding area following each trial. To begin a trial, a rat was placed in the start box, and the divider lifted. A white Plexiglas panel divided the opposite third of the arena into two sections. At testing, a terracotta digging bowl (internal rim diameter 7 cm, depth 6 cm) was placed in each section. Each bowl was defined by a pair of cues along two stimulus dimensions, the material with which the pot was filled and an odour (see Table 1). To mark each pot with a distinct odour, two drops (20 μl) of scented aromatic oil (Frontier Natural Brands, USA) was applied to the inner rim 5 d before use, and 3–5 μl reapplied the day before use. The bait, a 1/4 Honey Nut Cheerio (General Mills Cereals, USA), was buried 2 cm below the surface of the digging medium in the ‘positive’ pot. At the beginning of each task, a small quantity of powdered Cheerio was sprinkled onto the medium in both pots to prevent rats from locating the bait by smell rather than learning the discrimination.

Digging was defined as vigorous displacement of the medium to retrieve the reward. Simply investigating the rim or surface of the medium with paws or snout without displacing material was not scored as a ‘dig’. Thus, rats could access tactile, visual and olfactory cues.

The procedure entailed 3 d.

Day 1: Habitation. Rats were trained to dig in the pots for food reward. Two unscented baited pots were placed in the home cage for a series of three exposures, 5 min each, in which the bait was covered with increasing amounts of sawdust. Once the rat was digging reliably, it was placed in the test arena for three trials to retrieve reward from both sawdust-filled pots.

Day 2: Training. Rats were trained on a series of simple discriminations (SDs), to a criterion of six consecutive correct trials. They first had to learn to associate the food reward with an odour cue (lemon vs. rosewood, both pots filled with sawdust). After

<table>
<thead>
<tr>
<th>Discrimination stage</th>
<th>Dimensions</th>
<th>Example combinations</th>
<th>(+)</th>
<th>(-)</th>
</tr>
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<tbody>
<tr>
<td>SD</td>
<td>Odour</td>
<td>Clove/Sawdust</td>
<td>Nutmeg/Sawdust</td>
<td></td>
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<tr>
<td>CD</td>
<td>Odour</td>
<td>Clove/Raffia</td>
<td>Nutmeg/Metallic Filler</td>
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<tr>
<td>Reversal 1 (R1)</td>
<td>Odour</td>
<td>Nutmeg/Raffia</td>
<td>Clove/Metallic Filler</td>
<td></td>
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<td></td>
<td>Medium</td>
<td>Rosemary/Wood balls</td>
<td>Cinnamon/Plastic beads</td>
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<tr>
<td>ID shift</td>
<td>Odour</td>
<td>Rosemary/Plastic beads</td>
<td>Cinnamon/Wood balls</td>
<td></td>
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<tr>
<td>Reversal 2 (R2)</td>
<td>Odour</td>
<td>Cinnamon/Wood balls</td>
<td>Rosemary/Plastic beads</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>Velvet/Citroneilla</td>
<td>Crepe/Thyme</td>
<td></td>
</tr>
<tr>
<td>ED shift</td>
<td>Medium</td>
<td>Velvet/Thyme</td>
<td>Crepe/Citroneilla</td>
<td></td>
</tr>
<tr>
<td>Reversal 3 (R3)</td>
<td>Medium</td>
<td>Crepe/Citroneilla</td>
<td>Velvet/Thyme</td>
<td></td>
</tr>
</tbody>
</table>

CD, Compound discrimination; ED, extra-dimensional; ID, intra-dimensional; SD, simple discrimination.

Representative example of stimulus pairs, and the progression through stages of the AST protocol. In this example, odour was the initial discriminative stimulus dimension, shifting to digging medium in the ED stage. For each stage, the positive stimulus is in bold, and is paired randomly across trials with the two stimuli from the irrelevant dimension.
reaching criterion, they then had to discriminate by the digging media (felt strips vs. shredded paper, no odour). All rats were trained using the same stimuli in the same order. Training stimuli were not used again in testing trials.

Day 3: Testing. Rats were tested on a series of discrimination tasks (see Table 1). When the rat reached criterion of six consecutive correct trials, testing proceeded to the next stage. The first stage was an SD, involving only one stimulus dimension. Half the rats in each group were required at this stage to discriminate between two odours, only one of which was associated with reward, with both pots filled with sawdust. The other half began by discriminating the digging media, with no odours applied to the pots (for the sake of clarity, the remainder of this description considers only the example beginning with odour discrimination). The second task was a compound discrimination (CD), where the second, irrelevant stimulus was introduced. Only one odour was associated with reward, as in the SD, but two different digging media were paired randomly with the odours. The third stage was a reversal of this discrimination (R1), in which the same odours and media were used, and odour was still the relevant dimension, but the negative odour from the previous stage was now positive (i.e. associated with the reward), and the positive odour from the previous stage was now negative. The fourth stage was an intra-dimensional (ID) shift, wherein odour was still the relevant dimension but all new stimuli were introduced. The fifth stage was a reversal of this discrimination (R2), in which the previously negative odour was now positive, as in R1. The sixth stage required an ED set-shift, in which new stimuli were again introduced, but this time the relevant dimension was also changed, e.g. the digging medium became the relevant dimension and odour was now irrelevant. Finally, the seventh stage was another reversal (R3), where the previously negative medium was now positive. Table 1 outlines the progression through these stages, and provides examples of the cue combinations used. The dependent measure was trials to criterion (TTC), the number of trials to reach criterion of six consecutive correct responses at each test stage.

Elevated plus maze (EPM) test

In exp 2, the EPM test was conducted as described previously (Bondi et al. 2008), the day after the AST. Rats were transported to the testing room and allowed to acclimate for 15 min. The EPM had four white plastic arms, 10 × 50 cm, oriented in the shape of a cross, intersecting at a 10 × 10 cm platform. Two closed arms situated opposite each other were enclosed by 48 cm walls, and the remaining two open arms had no walls. The open arms were fitted with a 0.5 cm clear plastic rim around the edges to prevent animals from falling off. The maze was elevated 100 cm from the floor, and testing took place under normal ambient overhead lighting (200 lx in the open arms) with 60 dB background white noise. To begin the 5-min test, a rat was placed in the centre platform facing the junction of a closed and open arm. Activity was tracked using ANYmaze software (Stoelting Co., USA). Measures included time, number of entries, and distance travelled in each area of the maze. Open to total ratios (OTR) for time and entries (open/open + closed) were calculated as indices of open-arm exploration. Total distance travelled was analysed as a measure of non-specific changes in locomotion independent of OTR. The maze was cleaned with 70% ethanol, then water, and dried completely before the next test.

Expt 1: duration of the cognitive effect induced by 14-d CIC stress exposure

Eighty-three rats were randomly assigned to two groups: control or 14-d CIC stress. Seven days before testing, food was restricted to 14 g/d. Separate groups of control and CIC-stressed rats underwent habituation, training and testing as above, at 3, 7, 14 or 21 d after the last cold exposure (n = 8–12 per group at each time-point).

Expt 2: effects of 2-wk vs. 5-wk CIC stress exposure

To compare the effects of 2-wk and 5-wk CIC stress, 23 rats were randomly assigned to two groups: CIC stress or control, then further subdivided into 2- or 5-wk treatment groups. CIC stress was conducted as above. Seven days before testing, food was restricted to 14 g/d. Following the last day of cold exposure, rats were habituated and trained as above, and testing was conducted 3 d after the last cold exposure. The beginning of chronic stress treatment was staggered such that animals from all groups were tested together. As there were no differences between the two control groups, these were pooled into a single unstressed control group, resulting in n = 7–8 rats in each of three groups (unstressed controls, 2-wk CIC, 5-wk CIC). The day following the AST, rats were tested on the EPM.
Expt 3: effects of chronic antidepressant treatment on the CIC stress-induced reversal learning deficit

Fifty-nine rats in two independent cohorts (n = 30 and n = 29, respectively) were assigned to two treatment groups, control or 5-wk CIC stress. These were each further subdivided into two chronic drug treatments, either Cit and its vehicle control group (n = 7–9/group), or Des and its vehicle control group (n = 7–8/group). Rats were exposed to 2-wk CIC stress as above. On day 15, they were anaesthetized (43 mg/ml ketamine, 1.4 mg/ml acepromazine, 8.6 mg/ml xylazine, in 1.0 ml/kg i.m.). Osmotic minipumps (model 2ML4, Alzet Corp., USA), containing citalopram hydrobromide (Shanco International, USA), or vehicle (10% ethanol in saline), were implanted intraperitoneally (i.p.). Cit was delivered at a dose of 20 mg/kg.d free base, and Des at 7.5 mg/kg.d free base. Rats were given antibiotic (penicillin G, 300 000 IU/ml/kg). Three days after surgery, CIC stress treatment resumed for the remainder of the 5-wk period. Seven days before testing, food was restricted to 14 g/d. Rats underwent habituation and training as above, with testing conducted 3 d after the last cold exposure. Chronic stress and drug treatments were staggered such that rats from all groups were tested together. Each drug group was compared to its respective vehicle control. Because the high drug concentrations required for chronic delivery by minipump exceeded their solubility in saline, 10% ethanol was used as vehicle. A pilot comparison showed no significant difference in performance on the AST of rats treated chronically by minipump with saline vs. 10% ethanol (F(1,5) = 0.82, p = 0.41).

A separate set of 56 rats were used to test acute drug effects. Two independent cohorts (n = 32 and n = 24, respectively) were assigned to two treatment groups, control or CIC stress, then subdivided into two acute drug treatments, either Cit and its vehicle control (n = 8 per group), or Des and its vehicle control (n = 6 per group). Because the cognitive deficits after 2- and 5-wk CIC stress were comparable, acute drug treatments were tested only in rats exposed to 2-wk CIC. After stress or control treatments, rats were habituated and trained as above. On the test day, they were taken through the SD and CD test stages, then received an i.p. injection of either Cit (5.0 mg/kg free base) or saline vehicle (1 ml/kg). Our previous study showed that this dose of Cit acutely improved reversal learning performance of CIC-stressed rats (Lapiz-Bluhm et al. 2009). Testing resumed on the reversal stage 30 min after injection. Other rats, treated and tested in the same manner, received either Des (5.0 mg/kg) or saline vehicle (1 ml/kg i.p.), 30 min before reversal testing. This acute dose of Des, slightly lower than doses we have used previously, was selected based on pilot results indicating that doses of ≥7.5 mg/kg hindered performance of CIC-stressed rats on the AST. Control rats given 7.5 mg/kg Des were mildly sedated and took longer to perform, but generally completed all stages of the test, while a number of CIC-stressed rats did not, sometimes refusing to dig for >4 h.

Data analysis

Investigators were blind to the treatment conditions of rats being tested. Mean trials to criterion on the SD task on the training day were first compared by ANOVA, to ensure that acquisition and general performance capability were equivalent between groups.

For expt 1, data collected at each time-point following termination of CIC stress were analysed by two-way ANOVA (stress x task), with repeated measures over task. For expt 2, data were analysed by two-way ANOVA (stress x task), with repeated measures. Data from the EPM were analysed by one-way ANOVA. In expt 3, effects of Cit and Des treatment were analysed separately by three-way ANOVA (stress x drug x task), comparing each drug group to their respective vehicle controls. For acute injections, data collected for SD and CD tasks prior to the injections were analysed by two-way ANOVA (stress x task). Performance on the reversal task (R1) following acute injections was then analysed using two-way ANOVA (stress x drug). For all analyses, where significant main effects or interactions were indicated, post-hoc comparisons were performed using the Newman–Keuls test. Significance was determined at p < 0.05.

In all experiments, rats were weighed before beginning CIC stress or the corresponding control period, and again on the day of testing. Mean body weight gain was analysed by one-way ANOVA in experiments with stress as the between-group factor, or by two-way ANOVA with stress and drug as between-group factors.

Only rats completing all stages of the AST were included in the final analyses. A rat was eliminated if it stopped responding, or failed to complete all test stages within 5.5 h of testing, as continuing to test beyond that time approached the transition into the dark phase of the light cycle. This resulted in elimination of three rats from expt 1; three rats from expt 2;
seven rats from the chronic drug treatment study in expt 3, and three rats from the acute drug treatment study. Animals so excluded were not included in the reported number of rats used.

**Results**

Table 2 shows mean body weight gain in each experiment. In general, CIC stress slowed body weight gain moderately, but this was significant in only a few cases: in expt 1, the 3-d post-CIC group; and in expt 3, the acute Cit study.

**Expt 1: duration of the cognitive effect induced by 14-d CIC stress exposure**

CIC stress had no significant effect on training ($F_{1,32} = 0.21, p = 0.65$), indicating that chronic stress did not impair acquisition or the ability to perform on the test in general. This was true for all groups, regardless of the time at which testing was conducted after the last cold exposure.

Replicating previous results (Lapiz-Bluhm et al. 2009), 14-d CIC stress significantly impaired reversal learning 3 d following the last cold exposure (Fig. 1). ANOVA indicated no overall main effect of stress ($F_{1,21} = 2.99, p = 0.09$), but a significant effect of task ($F_{1,22} = 11.31, p < 0.001$), and a significant stress × task interaction ($F_{1,22} = 2.38, p = 0.03$). Post-hoc analysis at day 3 showed that CIC-stressed rats required significantly more trials to reach criterion selectively on the R1 reversal stage compared to unstressed controls ($p < 0.01$). However, similar analyses performed on data collected at days 7, 14 and 21 following the last cold exposure showed no significant differences in performance between controls and CIC-stressed rats on any task (Fig. 1).

**Table 2. Mean body weight gain at testing (weight at testing – starting weight)**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group</th>
<th>Body weight gain (mean ± S.E.M.), g</th>
<th>$F$ value</th>
<th>($^* p &lt; 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1</td>
<td>3 d post-CIC</td>
<td>Control: 18.67 ± 5.80</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>CIC: 14.33 ± 3.77</td>
<td>$F_{1,12} = 4.70^*$</td>
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<tr>
<td></td>
<td>7 d post-CIC</td>
<td>Control: 22.36 ± 6.55</td>
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<tr>
<td></td>
<td></td>
<td>CIC: 18.33 ± 9.02</td>
<td>$F_{1,11} = 1.48$</td>
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<tr>
<td></td>
<td>14 d post-CIC</td>
<td>Control: 26.25 ± 2.92</td>
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<td></td>
<td></td>
<td>CIC: 22.80 ± 2.81</td>
<td>$F_{1,16} = 0.80$</td>
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<tr>
<td></td>
<td>21 d post-CIC</td>
<td>Control: 36.63 ± 2.72</td>
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<tr>
<td></td>
<td></td>
<td>CIC: 31.50 ± 1.63</td>
<td>$F_{1,16} = 3.22$</td>
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<tr>
<td>Expt 2</td>
<td>2-wk CIC</td>
<td>Control: 18.75 ± 14.48</td>
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<td></td>
<td></td>
<td>CIC: 11.63 ± 10.51</td>
<td>$F_{1,18} = 0.18$</td>
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<td></td>
<td>5-wk CIC</td>
<td>Control: 91.50 ± 10.74</td>
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<td></td>
<td></td>
<td>CIC: 88.86 ± 9.75</td>
<td>$F_{1,9} = 0.04$</td>
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<tr>
<td>Expt 3</td>
<td>Chronic Cit</td>
<td>Control-Veh: 65.33 ± 4.27</td>
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<td></td>
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<td>Control-Cit: 59.71 ± 8.56</td>
<td>$F_{1,34} = 0.02$ (drug)</td>
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<tr>
<td></td>
<td></td>
<td>CIC-Veh: 52.71 ± 7.58</td>
<td>$F_{1,34} = 1.74$ (stress)</td>
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<td></td>
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<td>CIC-Cit: 56.86 ± 4.57</td>
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<td></td>
<td>Chronic Des</td>
<td>Control-Veh: 61.25 ± 5.45</td>
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<td></td>
<td></td>
<td>Control-Cit: 64.86 ± 4.48</td>
<td>$F_{1,35} = 0.04$ (drug)</td>
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<td></td>
<td></td>
<td>CIC-Veh: 57.00 ± 6.58</td>
<td>$F_{1,35} = 1.93$ (stress)</td>
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<td>CIC-Cit: 55.29 ± 4.48</td>
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<tr>
<td></td>
<td>Acute Cit</td>
<td>Control: 14.63 ± 3.43</td>
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<tr>
<td></td>
<td></td>
<td>CIC: 5.63 ± 3.86</td>
<td>$F_{1,30} = 4.33^*$</td>
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<tr>
<td></td>
<td>Acute Des</td>
<td>Control: 6.00 ± 5.21</td>
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<tr>
<td></td>
<td></td>
<td>CIC: 3.33 ± 3.47</td>
<td>$F_{1,22} = 0.20$</td>
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</tbody>
</table>

CIC, Chronic intermittent cold; Cit, citalopram; Des, desipramine; Veh, vehicle.

$^* p < 0.05$ compared to the corresponding control group.
Expt 2: effects of 2-wk vs. 5-wk CIC stress exposure

There was no difference in training of CIC-stressed rats and controls ($F_{3,19} = 1.92, p = 0.16$). Figure 2 shows the test-day performance of 2- and 5-wk CIC-stressed rats compared to unstressed controls. ANOVA revealed significant main effects of stress ($F_{2,20} = 6.49, p < 0.01$) and task ($F_{6,120} = 18.22, p < 0.001$), and a stress x task interaction ($F_{12,120} = 2.45, p < 0.01$). Post-hoc analyses again showed a significant deficit on the R1 reversal task, in both 2-wk ($p < 0.001$), and 5-wk ($p < 0.01$) CIC-stressed rats. Although in this experiment, the trials to criterion on the R1 task were significantly higher in the 2-wk stress group than in the 5-wk stress group ($p < 0.05$), the reversal deficit at 5 wk was comparable to that after 2 wk in expt 1, and in previous studies (Lapiz-Bluhm et al. 2009).

On the EPM, CIC stress induced no anxiety-like changes in open-arm exploration (Fig. 3), analysed as OTR for time ($F_{2,20} = 1.42, p = 0.27$), or entries ($F_{2,20} = 0.77, p = 0.48$). There were also no non-specific locomotor effects ($F_{2,20} = 1.29, p = 0.30$ for total distance travelled, not shown).

Expt 3: effects of chronic and acute antidepressant treatment on the CIC stress-induced reversal learning deficit

Neither stress ($F_{1,53} = 0.42, p = 0.51$) nor drug treatment ($F_{1,53} = 0.70, p = 0.50$) affected training. Figure 4 shows performance on the AST following 5-wk CIC stress exposure, with concomitant drug treatment during the final 3 wk. In the Cit experiment, ANOVA revealed significant effects of stress ($F_{1,58} = 4.42, p = 0.045$), drug ($F_{1,58} = 9.82, p < 0.01$) and task ($F_{6,348} = 10.43, p < 0.001$), with significant interactions between stress x drug ($F_{1,58} = 12.84, p < 0.01$), and stress x drug x task ($F_{6,348} = 3.05, p < 0.01$). As in the preceding...
Experiments, post-hoc analyses again revealed a CIC stress-induced deficit in reversal learning, with a significant increase in trials to criterion on the R1 task compared to vehicle-treated unstressed controls (p < 0.001). Chronic Cit treatment significantly reversed the CIC-induced impairment on the R1 task (p < 0.001, Fig. 4a).

In the chronic Des experiment, ANOVA revealed significant main effects of stress (F\textsubscript{1,28} = 8.71, p < 0.01) and task (F\textsubscript{2,56} = 9.21, p < 0.01), but no effect of drug (F\textsubscript{1,28} = 0.03, p = 0.86). There was a significant stress \times task interaction (F\textsubscript{2,56} = 5.90, p < 0.01). As above, post-hoc analysis showed that CIC stress significantly increased trials to criterion on the R1 reversal task (p < 0.001, Fig. 4b). In this experiment (but not in any others), there was a significant effect of CIC stress on the ED set-shifting task (p < 0.05). Neither of the CIC stress-induced deficits were reversed by chronic Des treatment (Fig. 4b).

In the acute drug study, there were no pre-existing differences in performance on the training day between rats in the drug and vehicle groups (Cit: F\textsubscript{1,28} = 0.96, p = 0.59; Des: F\textsubscript{1,28} = 0.81, p = 0.11). On the test day, there were also no differences between either of the drug groups and their respective vehicle control groups in performance on the SD and CD tasks before drug administration (Cit: F\textsubscript{1,28} = 0.005, p = 0.99; Des: F\textsubscript{1,28} = 0.24, p = 0.62). Analysis of the R1 reversal task after acute CIC treatment showed significant main effects of stress (F\textsubscript{1,28} = 8.93, p < 0.01) and drug (F\textsubscript{1,28} = 6.64, p < 0.05), but no interaction (F\textsubscript{1,28} = 2.60, p = 0.12). Post-hoc comparisons showed that CIC stress increased trials to criterion on the R1 task, and acute Cit significantly reduced trials to criterion on the reversal task in CIC-stressed rats, to a level comparable to that of unstressed controls (Fig. 5a), replicating our previous results (Lapiz-Bluhm et al. 2009). By contrast, acute Des treatment had no such effect (Fig. 5b). ANOVA showed significant effects of stress (F\textsubscript{1,28} = 9.91, p < 0.01), but no effect of drug (F\textsubscript{1,28} = 0.46, p = 0.51), nor a stress \times drug interaction (F\textsubscript{1,28} = 2.27, p = 0.15).

**Discussion**

Executive dysfunction related to prefrontal cortical dysregulation, specifically perseveration, or the failure to alter behaviour in response to environmental feedback, is prominent in depression (Channon, 1996). Cognitive behavioural approaches (Beck, 1976, 2005) presume that depression is associated with ruminative perseverations within a set of negative perceptions and beliefs about the self, the world and the future. The intent is to help patients challenge such biases, establishing a more flexible, realistic and adaptive cognitive schema based on hypothesis-testing and use of objective evidence to modify thoughts and behaviours. An animal model that reflects cognitive functioning of prefrontal cortex, is sensitive to stress, and is modulated by monoaminergic neurotransmitters and antidepressants, would be a valuable tool.
for investigating mechanisms involved in the dysregulation and restoration of cognitive flexibility in depression.

Results of the present study replicated our previous finding that CIC stress induces a selective impairment in reversal learning in rats, without interfering with acquisition of new contingencies. There were no differences in training, SD, CD or ID set-shift tasks. Moreover, as reported previously, CIC stress did not generally affect ED cognitive set-shifting (except in one experiment, see below), despite the rats having greater difficulty mastering the reversal task earlier in the test sequence, further demonstrating the relative selectivity of the CIC stress effect.

The deficit in reversal learning after 2-wk CIC treatment was transient. It was evident when tested 3 d after the last cold exposure, but had dissipated at time-points further past the last cold exposure, i.e.
at 7, 14, or 21 d. This was not of sufficient duration to investigate the effects of chronic antidepressant drug treatment initiated after the stress-induced cognitive deficit had been established, as in the clinical treatment of depression. Thus, in exp 2, the feasibility of continuing chronic stress treatment for a time that would be sufficient to allow chronic drug intervention was investigated. Five-week CIC stress induced a reversal learning deficit comparable to that after 2 wk. Thus, the 5-wk stress protocol was used in exp 3, with chronic antidepressant drug treatment beginning after 2 wk, when the reversal deficit had been established, and maintained for 3 wk thereafter, while CIC stress continued. This design is more relevant to the clinical treatment of depression, allowing chronic drug treatment to be initiated after the pathological behavior has been established, but also because treatment is maintained in the presence of continuing stress, as depressed patients undergoing antidepressant therapy will undoubtedly continue to experience many of the same life stressors that existed prior to the initiation of treatment. In this experiment, chronic treatment with the SSRI, Cit, but not the selective NE reuptake blocker, Des, effectively reversed the CIC stress-induced cognitive deficit.

Reversal learning, wherein a previously positive discriminative stimulus becomes negative, and a previously negative stimulus becomes positive, has been studied as a measure of cognitive flexibility in humans (Fellows & Farah, 2003; Hornak et al. 2004; Murphy et al. 2002; Rogers et al. 2000; Rolls et al. 1994), nonhuman primates (Clarke et al. 2004, 2005, 2007; Dias et al. 1996; Lee et al. 2007), and rats (Birrell & Brown, 2000; Boulougouris et al. 2007, 2008; Idris et al. 2005; McAlonan & Brown, 2003; van der Meulen et al. 2007). Reversal learning requires integrity of the OFC (McAlonan & Brown, 2003). Thus, the present results suggesting that reversal learning is sensitive to chronic stress may provide insight into how chronic stress alters prefrontal cortical function in ways that are relevant to the cognitive deficits in depression. Human studies have shown that acute cold exposure degrades cognitive performance, including vigilance, reaction time, reasoning and short-term memory (see Coleshaw et al. 1983; Mahoney et al. 2007; Patil et al. 1995; Shurtleff et al. 1993). Cold stress impaired performance on four-choice reaction time and delayed match-to-sample tasks, and increased measures of tension, confusion and depression, as well as ‘total mood disturbance’ scores (Mahoney et al. 2007). Limited studies of chronic cold exposure also suggest adverse effects on both cognition and mood (Makinen, 2007).

Food deprivation and changes in body weight alone have been reported to influence cognition in rats, although the evidence is equivocal. Food restriction for 3 months promoted long-term recovery of learning and memory following global ischaemia (Roberge et al. 2008a, b). However, 1-yr food restriction had negative effects (Yanai et al. 2004), and 40% caloric restriction for 6 months had no effects on cognition (Martin et al. 2007). In the present study, the relatively mild CIC stress treatment only moderately slowed body weight gain. CIC-stressed rats and controls had comparable performance during training, and on all cognitive tasks except the reversal task, and there were no differences in the effect of CIC stress on weight gain that could explain the beneficial effects of SSRI treatment. Thus, it is unlikely that differences in body weight alone could account for either the detrimental effects of CIC stress on reversal learning, or the beneficial effects of SSRI treatment.

We and others have also shown that reversal learning is specifically modulated by 5-HT neurotransmission in OFC (Clarke et al. 2004, 2005, 2007; Lapiz-Bluhm et al. 2009). In our previous study, the CIC stress-induced impairment in reversal learning was mimicked by 5-HT depletion, and was attenuated by acute administration of Cit, as in the present study. Together with a decrease in extracellular 5-HT levels measured by microdialysis in OFC during testing, these findings indicated a possible dysregulation of serotonergic modulatory function in the OFC of rats exposed to CIC stress (Lapiz-Bluhm et al. 2009). The present study provides further evidence that 5-HT facilitates reversal learning, in that chronic Cit treatment reversed the detrimental effect of CIC stress, even though stress exposure continued during drug treatment.

By contrast with Cit, the selective NE reuptake blocker, Des, had no effect on the CIC stress-induced reversal learning impairment, acutely or chronically. Anecdotally, as noted in the Methods section, CIC-stressed rats seemed more sensitive to the noncognitive, locomotor-inhibitory effects of acute Des. At doses that did not hinder performance of controls in completing the AST, CIC-stressed rats remained inactive, although seemingly alert, for several hours post-injection. Further experimentation would be required to investigate mechanisms of any possible increase in sensitivity to certain effects of Des, but these observations may be consistent with previous observations indicating that CIC stress can sensitize the response of subcortical noradrenergic receptors to NE (Ma & Morilak, 2005).

In the present experiments, only the first reversal task (R1) was consistently affected by CIC stress,
whereas the second (R2) and third (R3) reversals were generally unaffected. Similarly inconsistent effects on the later reversal tasks have been reported in other contexts (e.g. Bondi et al. 2008; Boulougouris et al. 2008; Hatcher et al. 2005; Tait & Brown, 2008). We have speculated that R1 may be more vulnerable to manipulation because of differences in the difficulty involved in reversing the contingencies established in the stages immediately preceding each of the reversals. R2 is similar to R1, as it follows a new acquisition within the same stimulus dimension, but it differs in that the animal has just had prior experience performing a reversal in R1. The ease with which rats typically master R2 may reflect a ‘learning-to-learn’ phenomenon, that may make R2 more resistant to disruption. By contrast, R3 is preceded by the ED set-shift, in which rats must abandon a cognitive set that had been reinforced repeatedly in all stages up to that point. Thus, the new contingency acquired in the ED task may not be as ‘strong’ as those established in earlier acquisitions, and less ‘flexibility’ may be required to achieve subsequent reversal in R3, making R3 less prone to disruption.

The selective reversal deficit after the chronic metabolic stressor in the present study was also different from cognitive effects reported previously after a more psychogenic stress treatment, CUS, which consistently induced ED set-shifting deficits, with less consistent effects on reversal learning (Bondi et al. 2008). CIC stress affected ED set-shifting in only one experiment in the present study. Moreover, chronic Des prevented the deficit in ED set-shifting induced by CUS (Bondi et al. 2008), but did not alter the effect of CIC stress on ED set-shifting in the present study, consistent with its lack of effect on the reversal deficit induced by CIC stress. Thus, it would seem that the prefrontal substrates affected by CIC stress are subtly different than those affected by CUS, and different types of stress may impact different forms of cognitive flexibility, dependent on different subregions of prefrontal cortex. CUS might produce effects that reflect functional changes in mPFC (see Bondi et al. 2008), while reversal learning, affected selectively by CIC stress, may reflect changes more specifically in functioning of OFC.

Further, CIC stress may be even more broadly useful for modelling other dimensions of depression as well. We have shown that CIC stress induced a dysregulation of the hypothalamic–pituitary–adrenal axis, as well as changes in NE release and postsynaptic adrenergic receptor sensitivity in stress-responsive subcortical brain regions, including the paraventricular nucleus of the hypothalamus (Ma & Morilak, 2005; Pardon et al. 2003). However, in the present experiment there was no anxiety-like reduction in open-arm exploration on the EPM after CIC stress, as was seen previously after CUS (Bondi et al. 2008). Moreover, some of the effects of CIC stress in previous studies were more pronounced in WKY rats, a genetically stress-vulnerable strain, compared to the more resilient Sprague–Dawley rats used in the present experiments. Thus, CIC as a model of metabolic stress might be useful specifically in addressing mechanisms underlying vulnerability to stress-induced behavioural and physiological pathology. Further research is required to understand how the behavioural, cognitive, physiological and affective dimensions affected by CIC stress might be related to each other, to the mechanisms underlying long-term consequences of chronic stress, or to differences in the efficacy of therapeutic interventions.

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Statement of Interest
None.

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