Neuropharmacology 70 (2013) 306–315

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

# Time-dependent effects of corticosterone on reward-based decision-making in a rodent model of the Iowa Gambling Task

Susanne Koot [a,b], Annemarie Baars [b], Peter Hesseling [b], Ruud van den Bos [a,c,*], Marian Joëls [a,*]

[a] Department of Neuroscience & Pharmacology, Rudolf Magnus Institute, University Medical Centre Utrecht, The Netherlands
[b] Division Behavioural Neuroscience, Department of Animals in Science and Society, Faculty of Veterinary Medicine, Rudolf Magnus Institute, Utrecht University, The Netherlands
[c] Department of Organismal Animal Physiology, Radboud University Nijmegen, The Netherlands

ARTICLE INFO

*Article history:*
Received 28 November 2012
Received in revised form
22 January 2013
Accepted 9 February 2013

*Keywords:*
Stress
Corticosterone
Rat
Iowa Gambling Task
Decision-making

ABSTRACT

Corticosteroid hormones, released after stress, are known to change neuronal activity in two time-domains: within minutes via non-genomic pathways and with a delay of >1 h through pathways involving transcriptional regulation. Recent evidence in rodents and humans indicates that these two modes of corticosteroid action differently affect cognitive tasks. Here, we investigated whether reward-based decision-making, in a rat model of the Iowa Gambling Task (rIGT), is also differently altered by rapid *versus* delayed actions of corticosterone. We targeted the rapid and delayed time domain by injecting corticosterone (CORT, 1 mg/kg, s.c.) at 30 min (rapid) or 180 min (delayed) respectively prior to behavioural testing, during the final 3 days of the behavioural paradigm. In saline treated rats, the number of visits to the disadvantageous arm decreased over trial blocks, whilst this was attenuated when CORT was administered 30 min before testing. This attenuation was associated with a significantly increased c-Fos expression in the lateral orbitofrontal cortex and insular cortex, and a trend for an increase in the infralimbic cortex. The rapid corticosteroid effect contrasted with treatment 180 min before testing, where the number of visits to the disadvantageous arm as well as c-Fos labelling was not affected. These findings indicate that rapid corticosteroid actions impair reward-based decision-making.


## 1. Introduction

Upon stress, corticosteroid hormones are released in high amounts. Shortly after stress onset, corticosteroids change neuronal cell function through non-genomic pathways (Joëls et al., 2012). Simultaneously a gene-mediated cascade is started which eventually will normalise increased neuronal activity. Several studies over the past years have shown that rapid (non-genomic) *versus* delayed (genomic) corticosteroid actions differentially affect cognitive function. For instance, through slow genomic actions stress improves spatial, declarative and working memory formation (Cornelisse et al., 2010; Henckens et al., 2011; Oitzl et al., 2001; Sandi and Pinelo-Nava, 2007; Smeets et al., 2009; Yuen et al., 2009). In the rapid time-domain, stress causes a shift from spatial and instrumental towards habitual learning strategies, in rodents as well as humans (Schwabe and Wolf, 2011). When targeting these two time-domains specifically by administering cortisol in human subjects either shortly or several hours before behavioural testing, working memory was found to be improved by slow compared to rapid corticosteroid actions, and this improved performance was linked to enhanced activity in the dorsolateral prefrontal cortex (Henckens et al., 2011). These and other studies have led to the hypothesis that prefrontal cortical functioning is impaired by corticosteroids acting via rapid non-genomic pathways, but enhanced by slow corticosteroid actions (Joëls et al., 2012).

One of the most important cognitive functions in daily life is decision-making. It comprises a complex assessment of short-term and long-term costs and benefits of competing actions. Recently, men were found to become more risk-taking under acutely stressful conditions in formal tests of risk-based decision-making, using e.g. the Iowa Gambling Task (IGT; Preston et al., 2007; van den Bos et al., 2009), the Balloon Analogue Risk Task (Lighthall et al., 2009) or the Game of Dice Task (Starcke et al., 2008; Starcke and Brand, 2012). In none of these studies, though, the two time-domains of corticosteroid actions were considered. We hypothesized that decision-making is impaired by rapid actions of corticosteroids but improved by delayed effects.

In the current study we tested this hypothesis in male Wistar rats, using a well validated rodent model of the IGT (rIGT; Homberg

* Corresponding authors. Department of Neuroscience & Pharmacology, Rudolf Magnus Institute, University Medical Centre Utrecht, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands. Tel.: +31 88 7568138.
*E-mail addresses:* ruudvandenbos1@gmail.com, ruudvdbos@science.ru.nl (R. van den Bos), m.joels@umcutrecht.nl (M. Joëls).

0028-3908/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuropharm.2013.02.008

et al., 2008; de Visser et al., 2011a, 2011b; van den Bos et al., 2006, 2012; van Hasselt et al., 2012; review: de Visser et al., 2011c), which assesses risk-based decision-making. In general, the output of decision-making processes is determined by an interaction between two different forebrain loops: *i*) a limbic (affective/motivational) loop, encompassing the orbitofrontal cortex, the amygdala and ventral striatum/nucleus accumbens, and *ii*) a cognitive (executive/motor) loop, encompassing the dorsolateral prefrontal cortex, anterior cingulate cortex and dorsal striatum (Bechara, 2005; de Visser et al., 2011a, 2011b, 2011c; Doya, 2008; McClure et al., 2004; Rivalan et al., 2011; Tanaka et al., 2004, 2007; Yin and Knowlton, 2006; Yin et al., 2008; Zeeb and Winstanley, 2011). While the limbic loop is involved in the early stages of the IGT, adjusting behaviour to changing contingencies, the cognitive loop is more involved in later stages, i.e. in future perspectives and cognitive control. The medial prefrontal cortex (mPFC) in rats has been suggested to share an anatomical and functional homology to the anterior cingulate cortex and dorsolateral prefrontal cortex in humans (Brown and Bowman, 2002; Uylings and van Eden, 1990; Uylings et al., 2003). We have previously shown in male Wistar rats, using transient inactivation of the mPFC with the GABA-agonists muscimol and baclofen in the last three daily sessions of the rIGT, that the mPFC becomes indeed more strongly involved as rats (express to) have learned task-contingencies, i.e. choose for the best long-term option (de Visser et al., 2011b). Therefore, in view of our hypothesis, CORT (1 mg/kg s.c.) was applied during the later stage of the task (final three daily sessions) either 30 or 180 min prior to behavioural testing, to probe the rapid and delayed effects on task-progression respectively. As corticosteroids are known to affect working memory and consolidation (Cazakoff et al., 2010; Roozendaal and McGaugh, 2011), we included parameters indicative hereof in our task. After the final session, c-Fos expression was determined, to examine the involvement of specific brain regions in the behavioural effects (de Visser et al., 2011a; van Hasselt et al., 2012).

## 2. Materials & methods

### 2.1. Animals

Male Wistar rats (Harlan, the Netherlands; Charles River, Sulzfeld, Germany; 9–11 weeks of age) were kept under controlled conditions (temperature 21 ± 2 °C, relative humidity 60 ± 15%) and a reversed 12 h light/dark cycle (lights off at 7:00 h or 8:00 h; depending on the interval between testing and corticosterone injections, see below). Rats were housed in pairs in Macrolon type-IV cages with a shelter and paper tissues as cage enrichments. Food and water were available ad libitum except during testing (see below). A radio provided background noise. All experiments were approved by the Animal Ethics Committee of Utrecht University and conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Directive 86/609/EEC).

### 2.2. Behavioural procedures

Experiments were run as described previously (de Visser et al., 2011a, 2011b) in order to keep the procedure similar to earlier used protocols. Therefore, after a habituation period of 2.5 weeks, all rats were first tested on the elevated plus maze (EPM) and subsequently in the rodent version of the Iowa Gambling Task (rIGT). Because anxiety, as measured by EPM performance, was earlier found to correlate with rIGT behaviour (Rivalan et al., 2009; de Visser et al., 2011a), we compared anxiety levels between batches of rats to promote that they were comparable prior to saline or corticosteroid treatment (see 2.5 Statistical Analyses). We refer to de Visser et al. (2011a) for detailed descriptions of the EPM protocol and analyses. We analysed percent time spent on the open arm as a measure of anxiety (de Visser et al., 2011a).

Rats were tested in the rIGT 1.5 week after EPM exposure (starting on Mondays; see de Visser et al., 2011a). The rIGT apparatus, made of grey PVC, consisted of a start box, choice area and four arms. Before testing, rats were habituated to the apparatus in a 10 min free exploration trial. Two days later they were mildly food deprived by providing for one day 50% of ad libitum food intake (on Sunday), followed by 30% of ad libitum food intake (for at least two days), which was subsequently individually adjusted to maintain rats at 90–95% of free feeding body weight during testing. Testing took place for 9 days, between either 9:00–12:00 h (lights off at 7:00 h) or 11:00–14:00 h (lights off at 8:00 h) for the rapid CORT/saline (30 min) and delayed CORT/saline (180 min) groups, respectively. Testing did not occur during weekend days. Food was freely available on these days, but rats were returned to their restricted diet the day before testing continued.

A trial started by lifting the slide door of the start box. Rats could freely enter the choice area of the apparatus and choose one of the four arms. To help rats differentiating arms, visual cues (10 × 10 cm; cross or circle in black or white) were placed to the side of the wall at the entrance of arms. When rats had entered a choice arm with their full body, including their tail, the arm was closed. At the end of the arm, rats could obtain pellets (baited arms) or nothing at all (empty arms). Each trial lasted max 6 min (inter-trial interval: 30 s). Rats received a total of 120 trials: 10 trials on days 1–6 and 20 trials on days 7–9. By the time rats reached the second half of the task, i.e. days 7–9, a session lasted 12 min at most.

Rewards were 45 mg sugar pellets (F0042, Bio-serv Inc, Frenchtown, NJ, USA); punishments were quinine-treated sugar pellets that were unpalatable but not uneatable. Rats were habituated to the sugar pellets in the week before the first rIGT session in their home cage daily, followed by a single session of providing two sugar pellets in a novel empty Macrolon type-III cage. Most rats consumed the quinine-treated sugar pellets once, but left them uneaten after briefly tasting them. Rats consistently eating quinine-treated sugar pellets were excluded from analysis. No animals were excluded because of this in the present experiments.

Of the four arms in the maze, two arms were baited and two arms were empty. The two empty arms were included to measure non-reward related exploration (de Visser et al., 2011a, 2011b, 2011c). The two baited arms consisted of a long-term disadvantageous arm and a long-term advantageous arm. In the disadvantageous arm, rats received occasional big rewards (three sugar pellets in 1 out of 10 trials) among frequent punishments (three quinine-treated sugar pellets in 9 out of 10 trials). In the advantageous arm, rats received frequent small rewards (one sugar pellet in 8 out of 10 trials) and infrequent punishments (one quinine-treated sugar pellet in 2 out of 10 trials). The positions of the baited and empty arms, as well as the advantageous and disadvantageous arm were counterbalanced across rats.

Performance parameters were: (1) the number of visits to the empty arms as a fraction of the total number of trials per block of 20 trials and (2) the number of visits to the disadvantageous arm as a fraction of the total visits to the baited arms per block of 20 trials. To unravel the potential mechanisms underlying behavioural effects of CORT injections, we measured several parameters in addition. First, for the disadvantageous and empty arms the average scores over the first and final 5 trials per block of 20 trials were compared as a measure of working memory, whilst the first 5 trials of a block of 20 trials were compared with the final 5 trials of the previous block of 20 trials as a measure of consolidation. Although these measures have not been validated in this particular task, they are comparable to approaches used in e.g. a radial maze (Butts et al., 2011; Conrad, 2010; Janitzky et al., 2011). Second, the total number of switches between different arms per block of 20 trials was calculated as measure of exploratory behaviour (de Visser et al., 2011a). Third, responses to encounters with sugar pellets or quinine-treated sugar pellets in the advantageous and disadvantageous arm were measured as win-stay/lose-shift behaviour per block of 20 trials (de Visser et al., 2011a). Blocks of 20 trials were chosen to obtain a sufficient number of encounters with sugar and quinine-treated sugar pellets. Thus, when rats encountered a sugar reward, their subsequent choice was scored as a win-stay when they revisited the same advantageous or disadvantageous arm. When rats encountered a quinine punishment, their subsequent choice was scored as a lose-shift when they switched to another arm. Win-stay and lose-shift behaviour were calculated as a fraction of the number of encounters with either sugar (win) or quinine (loss). Thus, values of 1 indicate win-stay and lose-shift tendencies only, while values of 0 indicate win-shift and lose-stay tendencies only.

### 2.3. Corticosterone injections

Saline or corticosterone (1 mg/kg CORT, stored at −20 °C until use; C174 Corticosterone HBC-complex, Sigma–Aldrich Inc.) injections were given subcutaneously on the last 3 days of the rIGT, either 30 or 180 min before testing. After injection rats were returned to their home cage, before they were tested in the rIGT. We chose to administer CORT s.c. in the second half of the task, i.e. the final three sessions of 20 trials each, because the mPFC becomes more strongly involved as rats have learned task-contingencies within the rIGT (de Visser et al., 2011b). The prefrontal cortex is particularly sensitive to the effects of stress exposure (Arnsten, 2009).

The dose of CORT was chosen to induce plasma CORT levels resembling those achieved after stress (de Quervain et al., 1998; Shafiei et al., 2012). To confirm this, the levels of plasma CORT were determined 30 min after CORT or saline injection in an additional group of rats that was exposed to the same experimental procedure (rIGT testing days 1–5), but from which blood samples were drawn through tail vein incision on days 6–9 instead of testing them in the rIGT. Levels of plasma CORT were not determined after 180 min as levels have been shown to peak after 30 min but return to nearly baseline within 90 min after 1 mg/kg (see e.g. Shafiei et al., 2012).

Blood was collected in heparinised (500 IU $ml^{-1}$) tubes and stored on ice. After centrifugation at 5000 r.p.m. for 10 min, the supernatant was stored at −20 °C until assay. Corticosterone plasma concentrations were determined with a