Neuroscience Research 70 (2011) 260–268

Contents lists available at ScienceDirect

Neuroscience Research

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

Behavioural and neural effects of diazepam on a rule-guided response selection task

Z. Muñoz-Torres[a], J.L. Armony[a,b], D. Trejo-Martínez[c], R. Conde[c], M. Corsi-Cabrera[a,*]

[a] Laboratory of Sleep, Facultad de Psicología, Universidad Nacional Autónoma de México, Av. Universidad 3004, Mexico City 04510, Mexico
[b] Department of Psychiatry and Douglas Mental Health University Institute, McGill University, Montreal, Canada
[c] Department of Magnetic Resonance, Hospital Ángeles del Pedregal, Mexico City, Mexico

ARTICLE INFO

Article history:
Received 27 August 2010
Received in revised form 6 March 2011
Accepted 20 March 2011
Available online 31 March 2011

Keywords:
Benzodiazepine
Diazepam
fMRI
Rule guided behaviour
Cognition
Drug
Human

ABSTRACT

Diazepam (DZ), a clinically important drug, reduces alertness and can interfere with complex cognitive processes. The effect of DZ on the behavioural and neural correlates of rule-guided response selection has not been directly investigated.

We studied DZ effects, compared to placebo (PL), on performance and brain responses, using fMRI, during rule implementation, when arbitrary stimulus-specific rules were involved.

BOLD activity was measured in eighteen healthy volunteers during rule-guided response selection with DZ or PL administered in two counterbalanced sessions.

A 10 mg dose of DZ was sufficient to increase reaction times and to reduce accuracy in a rule-guided task but not in a motor task containing the same stimuli. With PL, implementing arbitrary rules activated right anterior cingulate/middle frontal gyri. Under DZ more brain areas were recruited during the task compared to PL, especially occipito-parietal cortices, as well as the left temporal lobe. For the congruent trials rules, more activity was observed in the right retrosplenial cortex when participants had taken DZ.

These findings indicate that DZ might disrupt the neural activity necessary to implement novel rules, supporting the notion that DZ influence on behaviour goes beyond perceptual and motor processes that can potentially compromise complex cognitive functions.

1. Introduction

Every day life confronts us with the need to select rapid responses between alternative, contextually appropriate choices, based on the combination of several, potentially inconsistent sensory cues and abstract rules. For example, we need to stop the car on a green traffic light if a person is crossing the street. The adjustment of the usual course of action to the new circumstances require more than just passive sensory processing, it demands an active conscious supervision of the actual context and thus require the participation of higher-order cognitive systems (Fuster, 2008). Several neuroimaging studies in humans have identified an extensive and widespread network involved in flexible rule-guided behaviour, with the prefrontal cortex playing a general role in rule representation and other areas activated depending on the type of rule being represented (Bunge et al., 2003; Christoff et al., 2009), such as occipito-temporal areas for visual categorization (Tanaka, 2003), medial temporal lobe for memory (O'Neil et al., 2009), parietal lobe for visuo-spatial processing (Corbetta and Shulman, 2002) and frontoparietal communication for selecting sensory stimuli derived from internal goals (Corbetta et al., 2000).

Diazepam (DZ), a benzodiazepine (BDZ) derivative drug, is usually prescribed to reduce anxiety. DZ facilitates the action of GABA, the main inhibitory neurotransmitter in the adult mammalian brain, by enhancing chloride influx through the $GABA_A$ receptor complex (Mohler and Okada, 1977; Squires and Braestrup, 1977). In addition to its anxiolytic action, DZ has sedative and hypnotic effects that reduce the vigilance level which can lead to undesired side-effects, including drowsiness, performance deficits in vigilance tasks, reduced psychomotor activity and attention, as well as anterograde amnesia (for reviews see Curran, 1991; Woods et al., 1992; Nutt and Malizia, 2001; Buffett-Jerrott and Stewart, 2002). Indeed, several studies have shown that benzodiazepines in general, and diazepam in particular, can affect performance and brain activity in a variety of tasks, ranging from those demanding low cognitive processes such as finger tapping (Curran et al., 1998; Mintzer et al., 2001) and saccadic eye movements (Masson et al., 2000), to others involving higher cognitive load, such as discrimination reaction time (Berchou and Block, 1983), digit substitution (Curran et al., 1998; Mintzer et al., 2001), digit cancellation (Curran et al., 1998; Fluck et al., 2001), attention (Coull et al., 1995; Fluck et al., 2001), memory (Curran et al., 1998; Coull et al., 1999; Mintzer

* Corresponding author. Tel.: +56 22 22 51; fax: +56 22 23 10.
E-mail address: corsi@servidor.unam.mx (M. Corsi-Cabrera).

0168-0102/$ – see front matter © 2011 Elsevier Ireland Ltd and the Japan Neuroscience Society. All rights reserved.
doi:10.1016/j.neures.2011.03.009

and Griffiths, 1999), planning (Coull et al., 1999; Deakin et al., 2004) and deductive reasoning (Pompéia et al., 2007).

Critically, the effects of DZ, at behavioural and neural levels, during a rapid response selection task based on abstract rules have not been explored. Given that DZ receptors are distributed throughout the brain (Abi-Dargham et al., 1995; Millet et al., 2006) including cortical areas participating in flexible behaviour according to abstract rules, such as lateral prefrontal, anterior cingulate, posterior temporal and parietal cortices (Bunge et al., 2003, 2005; Christoff et al., 2009), and that inhibitory effects on brain metabolic activity in several of these regions have been reported during resting conditions (Veselis et al., 1997; Reinsel et al., 2000), we hypothesized that DZ would impair rapid response selection based on abstract rules.

In the present study, we investigated the effects, using functional magnetic resonance imaging (fMRI), of an acute single dose of 10 mg of DZ on the ability to select a response between alternative options in a rule-guided task containing arbitrary rules based on the combination of visual stimuli. Specifically, the task required the selection of appropriate and fast responses to compound stimuli according to rules which arbitrarily followed or contradicted the usual meaning of the information conveyed by the individual stimuli. To do so, we conducted a repeated-measures experiment in which the subjects were administered DZ or placebo (PL) in a counterbalanced manner prior to performing the rule-guided task in the scanner. We hypothesized that DZ-enhanced inhibition would affect neural processing of the stimulus-rule association, particularly at the prefrontal cortex, resulting in an impairment in performance.

2. Materials and methods

2.1. Subjects

Twenty healthy volunteers (10 men) participated in this study. Subjects ranged in age from 21 to 35 years (mean = 25.5) and had a body mass index of 23.4 ± 2.9 kg/m^2 (mean ± SD). Subjects reported having completed 14–21 (mean = 16.6) years of education. None of them had a history of head trauma or surgery, mental illness or drug addiction, and were free of medications known to affect the central nervous system or the DZ metabolism as determined by medical history and personal interview. Subjects began to refrain from taking alcohol or any medication 72 h before the recording sessions. All subjects were right-handed as assessed by Annett's test (1967). The study was approved by the Faculty of Medicine Ethics Committee of the National Autonomous University of Mexico and informed consent was obtained from all participants prior to the study.

Subjects ingested one of two similarly looking capsules containing either 10 mg of DZ or PL in a repeated measures counterbalanced design. Subjects were blind to the contents of the capsule. Scanning was performed between 2 and 5 h after the capsule intake, in a period when the DZ distribution has been reported as stable (Friedman et al., 1985). All participants underwent two experimental sessions separated by 3–5 weeks. Women were scanned during the early follicular phase (between 3rd and 5th day of hormonal cycle) to avoid potential confounding effects due to the interaction between DZ and sex hormones (Romano-Torres et al., 2002). Progesterone and estrogen levels were assessed and only women that presented plasma levels corresponding to the early follicular phase were included (estradiol 41.2 mcg/ml (SD 36.3); progesterone 1.33 mcg/ml (SD 3.2)).

Arterial blood samples were obtained just before or after fMRI scanning (counterbalanced) to determine DZ concentration by high performance liquid chromatography. Participants were conducted to their homes at the end of the sessions.

Data from two subjects (one man and one woman) were removed from the analyses due to technical problems with the stimulus presentation device.

2.2. Cognitive task

During scanning, subjects performed a reaction-time rule-guided task, based on stimulus-dependent complex arbitrary rules. The task required subjects to select between two alternative responses, to press a key once (single click) or twice (double click) with the right index finger as fast as possible, depending on a set of arbitrary rules. Following a preparatory screen with a central fixation point and two lateral squares, a white arrow appeared at the center of the screen for 500 ms, which could point to either side, immediately followed by the target (a red or green cross), which appeared in one of the squares for 100 ms. Thus, there were four different stimuli, which, critically, were unequal in their congruency with expected prepotent responses. Specifically, responses to the target when the arrow was pointing towards its location should be facilitated compared to when they were pointing away from it ("Posner effect") and responses to a green target should be faster than to a red one, based on the usual meaning of green ("go") and red ("stop") signals. Therefore, the *arrow towards green cross* (fully congruent: CC) was congruent in both dimensions, the *arrow towards red cross* (color incongruent: CI) and *arrow away from green cross* (direction incongruent: DI) were incongruent in one dimension (cross color and arrow direction, respectively), and the *arrow away from red cross* (fully incongruent: II) was incongruent in both dimensions. The fully congruent and incongruent stimuli required a double click whereas the partly incongruent one required a single click (see Fig. 1b).

Stimuli were presented in a pseudo-random order with the constraint that no more than three stimuli of the same type appeared consecutively. Four blocks of 40 stimuli were presented (10 of each of the four rules). Four additional "catch trials", in which no target followed the arrow and no response was required, were also presented in order to achieve a stochastic distribution of stimulus onset asynchrony and to provide an interstimulus baseline. An interval of 4000 ms separated the trials (see Fig. 1a). Reponses were recorded with an MR-compatible mouse. The task was run inside the scanner with E-Prime (Psychology Software Tools, Pittsburgh, Pennsylvania).

Subjects received the instructions and practiced the task outside the scanner before each fMRI session to avoid possible long term memory effects and to ensure the instruction for each rule was correctly understood. The practice test consisted in one block with 20 stimuli, 5 of each rule type; subjects performed the practice test until they got more than three correct responses of each rule type. Only 3 of the 18 subjects needed a second block to achieve this criterion.

In order to assess the DZ effects over general motor output, subjects performed a motor control task, containing exactly the same stimuli as in the cognitive task previously described, but were required to respond with a double click as fast as possible anytime the target (cross) appeared, independently of its color and location relative to the arrow.

The motor and cognitive tasks used here were previously tested in an independent, larger sample of subjects ($N = 39$). Results demonstrated significantly slower reaction times (RTs) for the cognitive task in comparison to the motor task and faster RTs for the fully congruent stimulus compared to the others, with no differences among those that had at least one incongruent component. Based on these findings (confirmed here; see Section 3), we divided the stimuli into *congruent* (or "easy") and *incongruent* (or "difficult") for the fMRI analysis.