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Optimization and pharmacological validation of a set-shifting procedure for assessing executive function in rats

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HIGHLIGHTS

- A widely used set-shifting protocol in a cross-maze was optimized for drug testing.
- The protocol was applied to rats treated by sub-chronic PCP administration.
- Potential reversal by tolcapone was also investigated.
- Overall, PCP-treated rats showed perseverative errors that could be reversed by tolcapone.

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ABSTRACT

Background: Set-shifting tests represent a reliable paradigm to assess executive functions in humans and animals. In the rat, set-shifting in a cross-maze is a recognized method. In this test, rats must learn an egocentric rule to locate food reinforcement. Once acquired, a second rule, based on visual-cue strategy, allows the location of the food. Ability of rats to shift from the first to the second rule is considered to reflect cognitive flexibility.

New method: This study aimed at optimizing the most currently used set-shifting protocol in a cross-maze for standardized drug testing by modulating the parameters related to caloric restriction, reward preference, and by redefining the notion of turn bias and classification of errors sub-types, *i.e.* perseverative *vs.* regressive. The new protocol has then been used to assess rats treated by sub-chronic phencyclidine administration and investigate the potential reversal effect of tolcapone, a brain penetrant catechol-O-methyl transferase inhibitor.

Results: The new procedure resulted in a decreased total duration and a re-definition of turn bias and error subtypes. Despite preferences for sweet rewards, caloric restriction had to be maintained to motivate animals. Overall, sub-chronic PCP-treated rats made mostly perseverative errors compared to controls and required more trials to shift between the two rules. Tolcapone partly reversed impairments observed in PCP-treated rats.

Conclusion: The new protocol has improved the reliability of key parameters and has contributed to the decrease of the test duration. PCP-treated rats submitted to this protocol have been shown to have significant deficits that could be reversed by tolcapone.

1. Introduction

Executive functions are described as a complex set of abilities, such as planning, solving problems, cognitive flexibility, that enable us to regulate efficiently behavior according to both external context and internal goals, particularly in unfamiliar situations (Eisenberg and Berman, 2010; Orellana and Slachevsky, 2013). Self-regulation of behavior through executive functions is strongly associated with a frontal-subcortical circuitry connecting different brain areas, including the prefrontal cortex identified as a key region (Alvarez and Emory, 2006; Jurado and Rosselli, 2007; Orellana and Slachevsky, 2013).

Deficit in executive functions is one of the core component of cognitive impairments in neurological and psychiatric diseases such as Parkinson's disease or schizophrenia (Godefroy, 2003; Hoptman and Nolan, 2009; Eisenberg and Berman, 2010; Millan et al., 2010; Orellana and Slachevsky, 2013). Behavioral

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flexibility in response to a change is commonly assessed in patients using the Wisconsin Card Sorting Test (WCST), a neuropsychological test dependent on prefrontal cortex normal functioning (Berman et al., 1995; Haut et al., 1996). Impairments in this test have been observed in schizophrenic and frontal-damaged patients and can be investigated in non-clinical species like non-human primates and rodents using set-shifting tasks (Dias et al., 1996). Various attentional set-shifting tests have been developed to study different aspects of cognitive flexibility in rodents through multiple stimuli dimensions. For example, Birrell and Brown (2000) proposed a procedure based on odor/medium/texture discrimination in bowls whereas some others proposed tasks involving a spatial/visual discrimination either in operant chambers or in a cross-maze (Ragozzino et al., 1999, 2002; Floresco et al., 2006a,b, 2008, 2009; Block et al., 2007; Ghods-Sharifi et al., 2008; Marquis et al., 2008). We decided to have a further look at the latter one.

The extra-dimensional set-shifting in a cross-maze was first described by Ragozzino et al. (1999) and Floresco et al. (2006) to assess executive functions in the rat. Only one procedure has been used ever since and was replicated by several laboratories with very slight modifications. Extra-dimensional shift learning consists, for rodents, in shifting between egocentric spatial and visual-cue response-based discrimination strategies. On the maze, rats are initially trained to make a 90° turn to receive food reinforcement. A visual cue is randomly placed in one of the choice arms on each trial but do not reliably predict the food location. Afterwards, during the set-shift, the rat is trained to use a visual-cue discrimination strategy and entering the correct arm by using the visual cue requires either a right or left turn. Thus, the rat must shift from the old strategy and approach the previously irrelevant visual cue in order to obtain reinforcement. The capacity of rats to shift strategy is considered a measure of behavioral flexibility, and is assessed by the number of trials and the number and type of errors made to learn the rule.

The first goal of the present study was to optimize the protocol proposed by Floresco et al. (2006) by establishing the conditions allowing reliable measurements of set-shifting for standardized drug testing in an industrial setting. The following parameters: caloric restriction, reward preference, length of daily session and definition of turn bias, as used by Floresco and colleagues, were reconsidered.

The new protocol has then been validated using a N-methyl-D-aspartate (NMDA) receptor antagonist-induced cognitive deficit as a model of hypofrontality in schizophrenia, namely phencyclidine (PCP) (Morris et al., 2005; Pratt et al., 2008). Descriptions of schizophrenia-like symptoms in healthy individuals and exacerbation of pre-existing symptoms in patients after repeated administrations of low doses of PCP have highlighted the involvement of disturbances in NMDA-dependent glutamate and dopamine transmissions in the pathogenesis of schizophrenia (Jentsch et al., 1997; Jentsch and Roth, 1999; Morris et al., 2005; Jodo, 2013). Sub-chronic PCP exposure in rats produces a pattern of neurochemical alterations, including glutamatergic, GABAergic and dopaminergic neurotransmissions, in the cortico-limbo-thalamic circuit, involving primarily the prefrontal cortex (Cochran et al., 2003; Morris et al., 2005; Wang et al., 2008). Cochran et al. (2003) showed that chronic intermittent low doses of PCP administered in rats led to a significant metabolic hypofunction in the prefrontal cortex, as well as other brain structures involved in the auditory system and within the thalamus. This dose regimen also produced a selective reduction of parvalbumin mRNA expression, reflecting the impairment of GABAergic inter-neurons, which directly modulate glutamate release and consequently impact on dopamine transmission downstream (Wang et al., 2008). This specific set of brain functional changes mimicked those reported for patients with psychosis, notably hypofrontality (Andreasen et al., 1997).

The potential effect of some dopaminergic modulators, such as the brain penetrant catechol-O-methyl transferase (COMT) inhibitor tolcapone, on cognitive processes mediated by the prefrontal cortex, has been explored in humans and in rodent models, and some beneficial impacts on a variety of cognitive tests were associated to its modulation of dopamine levels (Khromova et al., 1997; Tunbridge et al., 2004; Apud et al., 2007; Apud and Weinberger, 2007; Lapish et al., 2009). However, to our knowledge, there is no study report showing efficacy of tolcapone in a set-shifting procedure. Therefore, the second goal of this study was to investigate the sensitivity of the new set-shifting protocol to cognitive deficit expected to be observed after sub-chronic PCP administration and to evaluate if tolcapone was able to reverse the PCP-induced deficit in behavioral flexibility.

2. Materials and methods

2.1. Subjects

Male Lister Hooded rats (Harlan, France), weighing 250–280 g (approximately 10 weeks of age) were group-housed, with *ad libitum* access to water and food until beginning of caloric restriction, in a temperature and humidity controlled animal facility (12 h:12 h light:dark cycle, with lights on at 6:00 a.m.). Rats were acclimatized to the animal husbandry for a period of 5 days before any manipulation (*e.g.* weighting of the animals every day, start of caloric restriction, *etc.*). Rats were caloric restricted to 48% of their daily food intake in order to reach 85% of their initial body weight after about 2 weeks (Fig. 1). All animal procedures were conducted in strict adherence to the European Union Directive 2010/63/EU and were approved by UCB ethical committee. Behavioral experiments were carried out between 9:00 a.m. and 4:00 p.m., in a sound attenuated and air-regulated experimental room.

2.2. Apparatus

Two identical four-arm cross-mazes were used. Each maze was made of 1.5-cm-thick poly-vinyl chloride (PVC) and painted white. The mazes were placed on a removable squared platform that was elevated 70 cm above the floor. Each arm was 60 cm long, 10 cm wide, with 30-cm high walls. A sliding door (30 cm long × 10 cm wide) was located at 10 cm from the end of each arm, creating a start box, and a small cylindrical food well (2 cm wide, 1 cm deep) was set on the bottom of the door. A fifth door was used to block one arm of the maze to form a «T» configuration. Each maze resided in a room measuring 4 m × 4 m. Each rat batch was assigned to one testing room for all testing steps including habituation trials.

2.3. Preference for palatable food reward

In an attempt to suppress caloric restriction, 12 *ad libitum* fed rats were given the choice between 6 different palatable foods during a 30 min trial repeated for 4 consecutive days. The palatable foods were laying in equal amount (equivalent to 2 sugar pills, 90 mg) on a home cage floor devoid of sawdust: sugar pills (BioServ®), chocolate cereal (Kellogg's coco pops®), chocolate pearls (Jacques Pearls®), crunch peanuts (Delhaize®), petal corn (De Halm®), wheat cereal (Kellogg's all bran®). The sequence of food tasting and the time spent to consume each reward were measured. The first two trials were considered as habituations to new foods. Food preference was assessed during the last two trials by the sequence at which rat fully ate each reward. Accordingly, a score from 6 to 1 was attributed to each palatable reward for each rat; a score of 6 being attributed to the food first eaten.