The Ultimate Intra-/Extra-Dimensional Attentional Set-Shifting Task for Mice

Diego Scheggia, Audrey Bebensee, Daniel R. Weinberger, and Francesco Papaleo

Background: Alterations in executive control and cognitive flexibility, such as attentional set-shifting abilities, are core features of several neuropsychiatric diseases. The most widely used neuropsychological tests for the evaluation of attentional set shifting in humans are the Wisconsin Card Sorting Test and the Cambridge Neuropsychological Test Automated Battery Intra-/Extra-Dimensional set-shift task (ID/ED). These tasks have proven clinical relevance and have been successfully adapted for monkeys. However, similar tasks currently available for rodents are limited, mainly because of their manual-based testing procedures. The current limitations of rodent attentional set-shifting tasks are hampering translational advances in psychiatric medicine.

Methods: To closely mimic the Cambridge Neuropsychological Test Automated Battery ID/ED task in primates, we present the development of a novel operant-based two-chamber ID/ED "Operon" task for mice.

Results: We show the ability of this novel task to measure attentional set shifting in mice and the effects of genetic and pharmacologic manipulations of dopamine and glutamate. In genetically modified mice with reduced catechol-*O*-methyltransferase activity there was selective improvement on extradimensional shift abilities and impairment of serial reversal learning. Chronic administration of phencyclidine produced a selective impairment of extradimensional shift while producing a generalized decrease in latency to respond.

Conclusions: We demonstrate that this novel ID/ED Operon task may be an effective preclinical tool for drug testing and large genetic screening relevant to the study of executive dysfunctions and cognitive symptoms of psychiatric disorders. These findings may help elucidate the biological validity of similar findings in humans.

Key Words: Attentional set shifting, catechol-*O*-methyltransferase, executive functions, novel apparatus, phencyclidine, reversal learning

Attentional set shifting is a measure of cognitive flexibility and executive functions (1). It refers to the ability to switch between arbitrary internal rules ("cognitive-attentional sets"). The most widely used neuropsychological tasks for the evaluation of this function in humans are the Wisconsin Card Sorting Test (WCST) (2,3) and the CANTAB Intra-/Extra-Dimensional set-shifting task (ID/ED) (4). These tasks have been used to identify specific cognitive abnormalities in a wide range of mental disorders including autism (5), schizophrenia (6), Parkinson's disease (7), obsessive-compulsive disorders (8), and attention-deficit/hyperactivity disorders (9). The clinical relevance and solid methodologic approach of the WCST and the ID/ED tests have attracted interest in preclinical research (10,11). Importantly, these tasks allow for the selective measurement of discriminative learning, reversal learning, and switching of attention within the same dimension (intradimensional shift [IDS]) and between different perceptual dimensions (extradimensional shift [EDS]) within the same subject. This distinction is crucial because a double dissociation or functional specialization between the lateral (in monkeys and humans)/medial (in rodents) and orbital regions of the prefrontal cortex (PFC) have been demonstrated. That is, whereas the orbitofrontal cortex is selectively involved in the reversal phases of these tasks, the lateral/medial PFC region governs the EDS stages (1,12,13).

Despite their validity and utility in humans and nonhuman primates (4), similar tasks currently available for rodents (14–18) still suffer from limitations that dampen advancements in translating knowledge from animal research to psychiatric diseases. For instance, in the current ID/ED tasks for rodents, the presence of food reinforcers inside the choice stimuli might result in an ambiguous interpretation of animal responses and potential bias in choice making (10). Moreover, current set-shifting tasks are manually intensive and time-consuming. These characteristics limit the throughput of testing and application to and large-scale genetic and/or drug-screening studies.

To overcome these limitations and enhance the potential translational applications of ID/ED shift paradigms in rodents, we present here the development of a novel two-chamber, operant-based task to test attentional set-shifting abilities in mice. This novel task closely mimics the ID/ED task used in primates and circumvents the problems of the earlier rodent versions. We also tested the effectiveness of this new task in studies involving genetically modified mice and pharmacologic manipulations. Specifically, we tested catechol-*O*-methyltransferase (*COMT*) knockout mutant mice because they are an established clinically relevant mouse model with effects on executive cognition that recapitulate the pleiotropic behavioral effects of *COMT* genetic modifications in humans (19,20). *COMT* knockout mice have not been testable in a previously available attentional set-shifting task (16), most likely because of the interaction between the high-stress components of this manual task and the anxiety-like behaviors of these mutant mice. Moreover, we used our novel task to test the effects of chronic phencyclidine

From the Department of Neuroscience and Brain Technologies (DS, FP), Istituto Italiano di Tecnologia, Genova, Italy; Clinical Brain Disorders Branch (AB, DRW), National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland; Lieber Institute for Brain Development (DRW), Johns Hopkins University Medical Campus, and Department of Psychiatry, Neurology, and Neuroscience and the Institute of Genetic Medicine (DRW), Johns Hopkins School of Medicine, Baltimore, Maryland; and Dipartimento di Scienze del Farmaco (FP), Università degli Studi di Padova, Padova, Italy.

Address correspondence to Francesco Papaleo, Ph.D., Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova, Italy; E-mail: francesco.papaleo@iit.it.

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(PCP), a prominent pharmacologic model of schizophrenia that produces cognitive executive dysfunctions related to PFC (21). Furthermore, to assess whether our novel automated setup is really a more effective translational ID/ED task compared with existing standard systems, we contrasted the outcomes of our paradigm with those obtained using a simplified operant-based task that has been proposed for testing set-shifting abilities in rats (22).

Methods and Materials

Subjects

Testing was conducted in male mice, 3 to 7 months old, C57BL/6J or *COMT* null mutant (*COMT*−/−), and their heterozygous (*COMT*+/−) and wild-type (*COMT*+/+) littermates (16). Distinct cohorts of naive mice were used for each experiment. See Supplement 1 for detailed descriptions on animal housing, apparatuses, statistics, food restriction, and testing procedures.

Novel Two-Chamber Operon ID/ED Task

See Figure 1 for details on the apparatus.

"Stuck-in-Set" ID/ED Paradigm. For habituation to the apparatus, in the first two days, mice were habituated for 60 minutes to the apparatus with only neutral stimuli (Habituation 1) and trained to move from one chamber to the other (Habituation 2). Any nose poke into the nose-poke holes resulted in a pellet delivery into the food receptacle. The next day, mice were trained to perform two randomly presented simple discriminations (e.g., between Velcro [3M, St. Paul, Minnesota] vs. film; light on vs. light off; peach vs. sage) so that they were familiar with the stimulus dimensions (Habituation 3). These exemplars were not used again. The mice had to reach a criterion of 8 correct choices out of 10 consecutive trials to complete this and each following testing stage. Performance was measured in all phases of all experiments using number of trials to reach the criterion; time (in minutes) to reach the criterion; time (in seconds) from breaking the photobeams adjacent to the automated door to a nose-poke response (latency to respond).

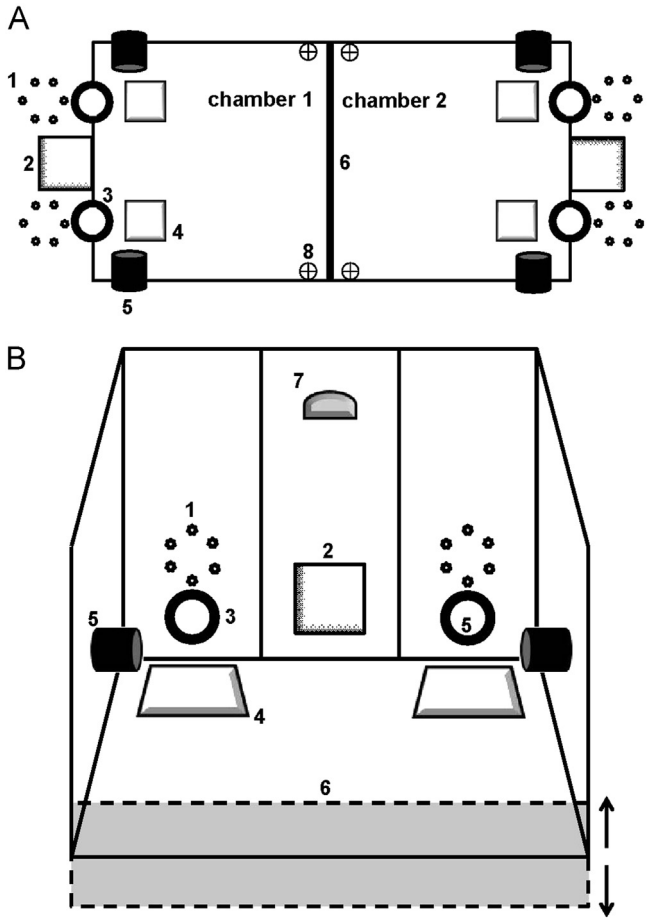

Figure 1. Schematic drawing of the novel two-chamber Operon apparatus. All procedures were approved by the Italian Ministry of Health and local Animal Use Committee and in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the European Communities Council directives. The apparatus consisted of two identical chambers with Plexiglas (Dobarca Srl., Genova, Italy) walls and aluminum floor, separated by an automatic transparent door that dropped to allow the mouse to change chambers. We used a two-chamber setup to exclude any possibility of postural mediation of the to-be-made response (27,46). Moreover, the two chambers also allowed us for automatic and continuous stimuli changes without interfering with the mouse. Each nose-poke hole was equipped with a series of changeable stimuli that could vary in three perceptual dimensions (odor, sight, and tactile; for stimulus exemplars used, see Table S1 in Supplement 1). We built this apparatus to allow for manipulation of three perceptual dimensions with a high number of stimuli for each dimension (at least 10 stimuli within each dimension). We devised stimuli with rodent-appropriate dimensions and a sufficient number of exemplars of each dimension to allow within-subject testing of all shifts, with novel stimuli at every shift. This total-change design eliminates confounding factors that can ambiguously affect the shift phases (47). Odors, lights, and textures were switched on or placed in the required position just before the start of a trial while the tested mouse was in the other chamber. **(A)** View from the top of the entire apparatus and **(B)** view from the front of a single chamber mimicking the mouse point-of-view during the test. 1, visual stimuli (light-emitting diodes); 2, food magazine; 3, nose-poke hole; 4, tactile stimulus (texture); 5, olfactory stimulus; 6, automatic sliding door; 7, house light; 8, infrared photo beam for door control. The apparatus was connected to a personal computer equipped with MED-PC IV software (Med Associates, St. Albans, Vermont). Chambers (16 × 16 × 16 cm) were separated by a transparent Plexiglas door (6). Infrared photobeams (8) tracked the animal movements and controlled the opening/closing of the automatic door to allow the mouse to change chambers. Each chamber presented two nose-poke holes (3) with infrared photo beams, and, between them, a food magazine (2) with photobeams delivering 14-mg rodent tablets (5TUL; TestDiet, St. Louis, Missouri). A fan and a house light (7) were located above each of the two food magazines. Each nose-poke hole was equipped with a series of changeable stimuli that could vary in three perceptual dimensions (odor, view, tact). For olfactory stimuli (5), liquid odorants were diluted in mineral oil (1:20; M5904, Sigma Aldrich, Dorset, United Kingdom) and presented on paper filter discs (15 uL, 2 cm) enclosed in metal pods placed on a rotating wheel mounted beside each nose-poke hole outside the chambers. Alternatively, we used two dilution olfactometers (PHM-275; Med Associates) that controlled the presentation of odor pairs inside the nose-poke holes (Figure S13 in Supplement 1). No differences were found in mice tested with both methods ($F_{1,14} = .00$; $p = .95$). Ten odor stimulus exemplars were used for both methods (Table S1 in Supplement 1). For visual stimuli (1), light-emitting diodes were placed on top of each nose-poke hole. Up to six color stimulus exemplars could be presented to the test subject. Light stimuli were switched on in pairs (red–green, yellow–blue, white–orange) and counterbalanced between left and right nose-poke holes (Table S1 in Supplement 1). For tactile stimuli (4), changeable floor textures, sliding under the floor, were mounted in front of each nose-poke hole. There were up to 10 texture stimulus exemplars (Table S1 in Supplement 1). Thus, the discriminative association between a correct response (which will result in food delivery) and a nose-poke hole could be varied by their odor, visual cue, or the floor texture. Stimuli and operant-based rewarding components were located in the same positions in chamber 1 and chamber 2. See also Figure S14 in Supplement 1 for a photograph of the apparatus.