

How Can a Touch-Screen Based Visual Discrimination Help to Better Characterize Rodent Models of Schizophrenia?

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Ethical statement

All animals were treated in accordance with the European Ethics Committee (decree 86/609/CEE), the Animal Welfare Act (7 USC 2131) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003). The study protocol was approved by the local animal experimental ethical committee at Janssen Research & Development (Beerse, Belgium).

Introduction

Existing in many forms, two of the most commonly used cognition paradigms in the rodent are rule acquisition and reversal learning (e.g. swim mazes, operant tasks, T-mazes). Despite the wide spread use of these simple assays, interpretation is often not straightforward and important controls are commonly overlooked. For example, when acquiring a simple discrimination (a response to stimulus “A” is rewarded, whereas a response to stimulus “B” is not), necessary for many forms of operant learning, the animal must learn several concepts. Rodents must (1) learn to distinguish the stimuli, (2) learn that only one of the stimuli is associated with reward, (3) and that earning a reward is dependent upon their own behavior. Accordingly an impairment in task acquisition could be caused by a failure in any one of these processes or other non-specific effects, like alterations in motoric abilities. Reversal learning has similar, but unique, difficulties in interpretation. In rodents, reversal learning paradigms can take days, and are often split into three distinct phases. Early reversal is the first stage of the process, occurring immediately after the switch in rule contingencies associated with reward. The rodent’s behavior is capable of earning a reward, but its current behavior is not; it must learn the new contingency. Until this occurs, the animal will consistently perform below chance. As it comes to understand this change, the animal will begin to experiment with other responses to see if they earn a reward (middle reversal), and their accuracy will begin to increase towards chance level performance. Finally, as the animal learns that it can still earn a reward, it must learn which stimuli is now associated with a reward, a stage called late reversal. Depending upon the stage reversal learning does require different neurobiological and psychological processes (for example see Bussey et al., 1997, Chudasama and Robbins, 2003), some of which may overlap with acquisition learning. Considering the numerous processes involved in acquisition of a discrimination, and the overlap between reversal learning and acquisition of a discrimination, it is important to understand the psychological process impaired, not just the stage impaired (i.e. visuo-perceptual changes will impair acquisition, but this does not mean the animal is impaired at learning).

To this end, we used touch-screen technology to develop the Double Visual Discrimination task (DVD). Using modified operant chambers animals are trained to nose-poke at “images” displayed upon a touch-sensitive computer monitor to earn a reward. The “rules” dictating this reward can be modified to fit the experimental question at hand and should help to dissociate the individual elements of rule learning, stimulus discrimination, recall and reversal learning, in one procedure. In the DVD procedure animals are first trained to perform a visual discrimination (learn that a response at image “A” results in a reward whereas a response at image “B” does not). When this has been acquired a second pair of images is introduced, however some trials are still given with pair 1. In this way animals must learn a second discrimination while still performing the first. Finally, once the second pair has been learned, the reward outcomes of pair 1 are reversed (see figure 1 for example baseline data). In this way the DVD procedure should be able to dissociate the cognitive processes affected by a manipulation, rather than just the gross behavior (recall, acquisition, reversal learning). Although this new paradigm can be applied to numerous disease models, in this instance we have selected acute PCP challenge. Often used as a model of schizophrenia, the NMDA receptor antagonist is also known to disrupt learning and other cognitive functions. While evidence suggests that PCP given acutely can influence acquisition of a task,

reversal learning, and baseline task performance, published studies do not use cohesive methodologies. As such, the comparative nature of these deficits, the concentration at which they occur, and the associated side effect profiles remain largely undescribed in a unified testing environment. Accordingly, we have performed dose response studies at each stage of the DVD procedure to elucidate the nature of PCP impairments on several cognitive processes using a unified testing procedure.

Materials and methods

Animals: Adult male Lister-Hooded rats (purchased from Harlan, The Netherlands) were used in this study (230- 250 g) at the beginning of the training). Access to food was restricted so that rats were maintained at 80-85% of their free-feeding body weight.

Apparatus: Testing was conducted in Med-associate chambers (internal dimensions: around L 33 cm x W 40.5 cm x H 29 cm) equipped with a touch screen (40 x 25 cm). A metal flap (36.5 x 5 cm) was located in front of the touch-screen, around 5 cm above the grid floor. A black metal insert was added on the touch-screen (1 cm from it) to create 4 response windows (8.2 x 15 cm). The wall located opposite to the touch-screen presents the food magazine (equipped with an infrared nose pokes detectors and a light), the loud-speaker and the house light. The testing apparatus was located in a sound-attenuating chamber (H: 60cm x W: 74cm x L: 60cm) ventilated by a fan. Stimuli and data recordings were controlled using K-Limbic software.

Pre-training: The experiment began with a short pre-training procedure during which the rats learn to vigorously respond to the touch-screen. This started with a tone training procedure where rats were taught to associate a “tone” with a delivery of a food reward. This was followed by a procedure where any response at the screen was rewarded while it was illuminated. When rats were reliably doing this they were advanced to the final stage of pre-training where only a portion of the screen would be illuminated and only responses at the illuminated portion were rewarded. A response at non-illuminated areas of the screen had no consequence.

The DVD task: The task itself takes place in three stages: (1) Simple Visual Discrimination, (2) acquisition of a second discrimination while still performing the first discrimination, and finally (3) the reversal of one of the previously learned pairs. During acquisition of the first stage a response at one image always results in a reward, whereas a response at the other image would lead to a short timeout (10sec) during which the house light was deactivated. Once the timeout period elapsed, the animal would attempt the same trial again, i.e. a correction trial. During this stage, training sessions were made of 48 trials or lasted 30 min whichever occurred first. Once animals had mastered this task and reached criterion (i.e. accuracy higher than 85% and standard deviation of less than 10 for at least three successive days) they were moved to the second experimental stage, the double visual discrimination. During this step, they were required to learn a second discrimination with new images, while also being presented with the original image pair. Both sets were presented but on separate trials and were randomly mixed across trials. Finally, after the second discrimination had also been mastered, the rats experienced a reversal where the contingencies associated with the first learned pair were reversed. Training stages with two sets of stimuli consisted of a total of 96 trials or lasted 60 minutes whichever occurred first. For a graphical depiction of this procedure, see figure 1.

Stimuli: In this paradigm, two pairs of stimuli were used: Flower/Bomb and Spider/Plane, each stimulus being ~5.5cm X 5.5cm size and highly similar to those used by Bussey et al. (1). The pair of stimuli presented during each experimental stage were counterbalanced between animals. For each pair of stimuli, only one stimulus was rewarded (S+) and the S+ of each pair being counterbalanced between rats. Importantly, the location of the S+ and S- were randomized over the four windows between trials. 4 windows were used to prevent the animal from seeing the two possible stimuli arrangements as a “scene” and responding using an “if ‘A’ go right, if ‘B’ left”, and to instead perform the task as a two-way discrimination.

The comparison between the acquisition of the first pair and acquisition of the second pair may allow the dissociation between procedural and stimuli learning, versus stimuli learning alone. Moreover, during acquisition of the second pair, the original pair also serves as a control for changes to “recall” or non-specific effects that

may disrupt cognition. Finally, the comparison between acquisition phases and reversal learning will help determine if any deficits observed are specific to reversal learning, or if they affect learning in general.

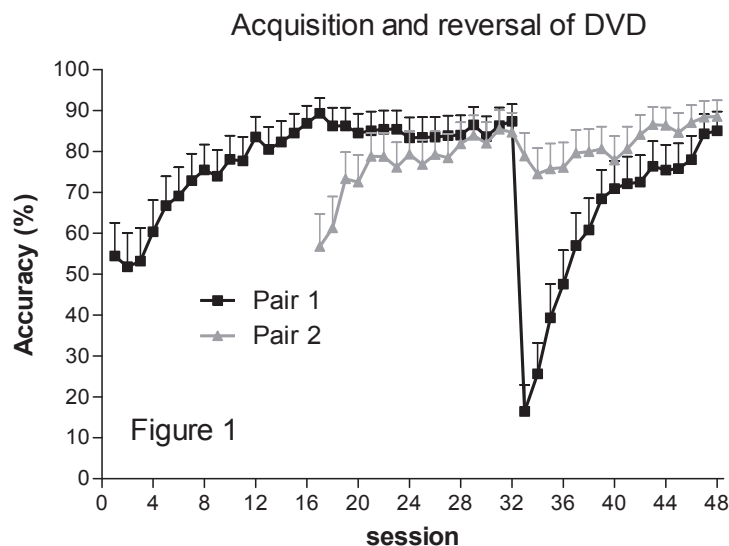
Treatment: Rats received either subcutaneous injections of phencyclidine (PCP group) or vehicle (vehicle group) 30 min before each training session. Solutions were administrated at 1ml/kg (s.c.).

Behavioral analysis: Animals' performance was recorded and collected automatically using K-Limbic software. For each training session the following measures were analyzed: the percentage of completed trials (number of trials completed /maximum number of trial*100; the mean accuracy (percentage of correct response relative to the number of completed trials), the mean response latency (i.e. time to select the correct/incorrect choice once the two stimuli are displayed on the screen) and the mean collection latency (i.e. time to collect the food reward in the food magazine once it has been delivered). Importantly, correction trials were not taken in consideration in the analysis of these parameters. They were however used to calculate correction trial accuracy, i.e. the number of correction trials required for an animal to get a correct response after it responded incorrectly to a trial.

Statistical analysis: Accuracy was analyzed using a non-linear regression model and complementary analysis was performed using a two-way ANOVA with treatment as the between group factor and training sessions as the within group factor. Dunnett post-hoc comparisons were made where appropriate, having the vehicle group as a control group. All other parameters were analyzed using two-way ANOVA as described above.

Results

Doses of PCP used in behavioral studies can often cause marked hyper-locomotion and impairments in motoric functions. However in these studies very low doses of PCP (0.25-1 mg/kg) were used in hopes that any effects observed would be the result of changes in "cognition" as opposed to non-specific side effects influencing the fundamentals of task performance. During acquisition 1, PCP caused dose-dependent disruptions in learning, with a modest effect seen at 0.5 mg/kg and a more robust impairment at 1.0 mg/kg. Importantly, no signs of motoric impairments were observed. In fact, animals had a tendency to respond faster when given PCP, likely a result of PCP's stimulant properties. On the second acquisition, significant impairments were seen at 0.5 mg/kg with minimal effects on the previously learned pair. These data suggest that PCP can disrupt image acquisition independently of rule learning. Finally, PCP also impaired reversal learning, while having minimal effects on the unchanged stimuli. These data indicate that while PCP may disrupt learning about stimuli, it is also capable of disrupting adaptation to new reward contingencies since the stimuli themselves do not change during reversal learning.



Conclusions

Using this procedure, we have demonstrated that PCP disrupts numerous aspects of cognition within a unified paradigm. Moreover, these impairments occur at doses lower than those often used in behavioral studies, and occur with minimal side effects. While PCP may disrupt reversal learning, it also impairs acquisition which should also be involved in reversal learning. As such a PCP challenge appears to impair task acquisition in general, as opposed to being specific to reversal learning, although more research is necessary to confirm this. Importantly, these effects on reversal learning and acquisition occurred with minimal effect on recall of a second pair of images suggesting that the effects seen are not the result of a global disruption.

Despite its popularity as a research tool, acute PCP treatment as a pharmacological tool to study cognition or as a model of psychosis has not been comprehensively described. Using the DVD procedure, we have described how low doses of PCP effect acquisition, reversal learning and task performance within a unified procedure. These data highlight the utility of the DVD procedure in allowing the simultaneous measurement of multiple cognitive processes within a single animal. The DVD procedure can be used to gain confidence in the selectivity of a specific change in cognition. Furthermore, DVD can also be applied to other behavioral models, and may be particularly effective at helping to describe the effects of chronic impairment models, be it sub-chronic PCP, early life stress, lesions, or transgenic animals, through the use of multiple acquisition and reversal phases. When combined with other tasks currently available for use within touch-screen equipped operant boxes, the DVD procedure should make an important contribution to the characterization of disease models and accurate interpretation of data.

Acknowledgements

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References

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