Cognitive performance and response inhibition in developmentally vitamin D (DVD)-deficient rats

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Abstract

Evidence from epidemiological studies suggest that low levels of vitamin D during early life alter brain development and may increase the risk of various adverse health outcomes, including schizophrenia. The aim of this experiment was to examine the effect of developmental vitamin D (DVD) deficiency on attentional processing using the 5-choice serial reaction time task (5C-SRT) and the 5-choice continuous performance test (5C-CPT), which specifically assesses sustained attention and vigilance in rodents. DVD-deficient and control rats were exposed to a series of target and non-target trials within each operant testing session. A number of measures were recorded including hit, miss, false alarm and correct rejection, as well as premature and perseverative responses. Performance on 5C-CPT was also assessed after administration of the atypical antipsychotic, clozapine. The adult offspring of DVD-deficient rats had higher levels of impulsivity, as demonstrated by a significant increase in premature responses. On the 5C-SRT and target trials of the 5C-CPT, accuracy was not significantly affected by prenatal diet; however DVD-deficient rats made 50% fewer correct rejections compared to controls on non-target trials of the 5C-CPT. Thus, control rats were able to discriminate between target and non-target trials, whereas DVD-deficient rats were unable to make this discrimination. Clozapine reduced the occurrence of false alarms in DVD-deficient rats to a level comparable to control values. Taken together these data suggest DVD-deficient rats have increased impulsivity as well as a lack of inhibitory control, and these features may be informative in terms of modeling the cognitive deficits observed in schizophrenia.

INTRODUCTION

Schizophrenia is a poorly understood but very disabling group of brain disorders affecting approximately 1% of the population [1]. While hallucinations and delusions (positive symptoms) feature prominently in diagnostic criteria, impairments on memory and attentional processing (cognitive symptoms) are attracting increasing interest in modern neuropsychiatry [2]. For example, data from prospective birth cohorts have shown consistent evidence that cohort members who develop schizophrenia as adults tend to have deficits in a range of premorbid intellectual impairments covering verbal, non-verbal and mathematical abilities and attentional, executive and motor impairments [3]. With respect to clinical outcomes, compared to positive symptoms, neurocognitive measures have been repeatedly found to have greater predictive value with respect to social and occupational recovery [4,5].

Based on clues from epidemiology, we have proposed that low prenatal vitamin D may be a risk factor for later development of schizophrenia [6]. Many studies have shown that those born in winter and spring have a significantly increased risk of developing schizophrenia [7], and that those born at higher latitudes are also at increased risk [8], with both the incidence and prevalence of schizophrenia being significantly greater at higher latitudes [9]. In a recent case-control study we have shown that low prenatal vitamin D is a plausible candidate risk factor for this disease, because those with low levels of vitamin D at birth had a two-fold increase in the risk of developing schizophrenia in later life [10].

Prenatal vitamin D deficiency has been shown to alter brain development and adult behaviour in rodents [11,12], in which the foetus develops in utero in a vitamin D-deficient but normocalcaemic dam. For example, developmental vitamin D (DVD) -deficient neonates had larger lateral ventricles, increased cell proliferation and reduced apoptosis [13] as well as altered dopamine ontogeny [14] and signaling [15]. Adult DVD-deficient animals had larger lateral ventricles compared to controls [16], as well as significant changes in protein and gene expression in the brain [17]. Behaviourally we have shown that DVD-deficient adults have a transient “hyperlocomotion” compared to controls [18-20] and they have impaired responses on tasks assessing latent inhibition [21]. Finally, DVD-deficient rats have enhanced locomotion in response to the NMDA antagonist MK-801 [22], a behaviour which is blocked by the antipsychotic, haloperidol [20].

The assessment of cognitive deficits in patients with schizophrenia can include a bewildering array of potential measures. Similarly, the assessment of attention, learning and memory in rodent models could follow many different directions [23]. The prominent test of attentional processing in rodents is the 5-choice serial reaction task (5C-SRT), which incorporates measures of impulsivity and perseverance [23,24]. Schizophrenia patients have repeatedly been shown to perform worse than controls on the continuous performance test (CPT) [25]. In alignment with the human CPT, the addition of a withhold condition has recently been incorporated into rodent testing resulting in the 5-choice continuous performance test (5C-CPT) [26].

Building on the 5C-SRT, response inhibition and vigilance can now also be assessed. Response measures used in signal detection theory such as hit, miss, false alarm and correct rejection can now be measured in rodents, creating a test that more closely mirrors the human CPT [26].

Based on the most robust findings in schizophrenia [27], and recommendation of the panel for the Measurement And Treatment Research for Improving Cognitive Symptoms in Schizophrenia (MATRICS) [28,29], the focus of the current study was to investigate cognitive functioning in the DVD-deficient rat. The DVD-deficient rat model is based on evidence from disease epidemiology and displays good face validity as a developmental animal model of schizophrenia. This study aimed to assess cognitive deficits in the DVD-deficient rat on the 5C-SRT and 5C-CPT, before investigating the effects of the atypical antipsychotic clozapine on performance. Antipsychotics generally have significant negative side effects and poor efficacy in the treatment of cognitive symptoms, however selective improvements in function have been reported in patients and animal models [30,31].

MATERIALS AND METHODS

Animals and Housing

All procedures were performed with approval from the Queensland University Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia. Sprague-Dawley rats (Herston Animal Facility, QLD, Australia) were housed in same-sex pairs in Macrolon cages

with Sanichip bedding and wire lids at a stable temperature of 21 ± 1 °C. For the 5C-SRT and 5C-CPT experiments, 16 DVD-deficient (n = 8 females, n = 8 males) and 12 control rats (n = 6 females, n = 6 males) were used from 20 weeks of age. Rats were reared, housed and tested at The University of Queensland and, prior to testing, had free access to food and water.

Vitamin D deficient and control offspring were generated as previously described [32]. Briefly, female Sprague-Dawley rats were kept on a vitamin D deficient diet (Vitamin D Deficiency AIN93G Rodent diet, Dyets Inc., Bethlehem, PA, USA). Animals were housed on a 12h light/dark cycle (lights on at 0600 h) using incandescent lighting, to avoid ultraviolet radiation within the vitamin D action spectrum. These conditions were maintained for six weeks prior to mating and throughout gestation. Control animals were kept under similar conditions except they received a vitamin D replete diet (Standard AIN93G Rodent diet, Dyets Inc., Bethlehem, PA, USA). At birth vitamin D deplete dams (and corresponding litters) were given a vitamin D replete diet and all rats were kept under the same conditions for the remainder of the experiment. At weaning (postnatal day 21) all animals were housed in same sex groups of 2 and kept on standard food (Feeder and Grower diet, Specialty Feeds, WA) until behavioural testing. Rats were placed on a restricted feeding schedule to decrease body weight to ~90% of their original free-feeding weight and remained food restricted throughout training and testing. Rats had ad libitum access to water in their home cage.

Testing Apparatus

Training and testing took place in eight 5-hole operant chambers (50x50x50cm, Med Associates Inc., St. Albans, VT, USA) situated in a room under dim red light. The chamber was housed in a ventilated, sound attenuated box and ventilated by a fan with background noise of 70dB presented from a speaker within the chamber. The rats were trained to respond to brief pulses of light (6.4 mm diameter yellow LED) within one of five equally spaced apertures (2.5 x 2.5 x 2.2 cm) situated within a curved aluminum wall. A nose poke into the aperture broke an infrared beam and this would result in delivery of a 45 mg food pellet (F0021, dustless precision pellet, Bioserv, Frenchtown, NJ, USA) to a food magazine located opposite to the 5-hole wall. The food magazine was fitted with a light (10 mm diameter white bulb) and head entries to the magazine were recorded using an infrared beam. A house light was located on the ceiling directly above the food magazine. The control of stimuli and recording of responses were managed by two SmartCtrl Packages 8-In/16-Out with additional interfacing by MED-PC for Windows (Med Associates Inc., St. Albans, VT, USA).

5C-SRT

Rats were trained to perform the 5-choice serial reaction task (5C-SRT) as described previously [33], with each session lasting 30 min or 100 trials, whichever was completed first. Briefly, rats were trained to detect a stimulus light in one of the 5 aperture locations with various parameters adjusted across training sessions including: duration of the stimulus light (30, 20, 10, 5. 2.5, 1.25, 1 s), limited hold period (30, 20, 10, 5 s) and reward duration (10, 5, 2 s), with a fixed inter-trial interval (ITI) of 5 s. We recorded correct and

incorrect responses within the limited hold time. Rats were given a 5 s time-out, when the house light came on, for an incorrect response, omission error or premature response. Repeated nose pokes to any aperture after the initial response was recorded as a perseverative response, which did not affect the duration of the time out. During the final phase of training the trials were separated by a variable ITI (3, 4, 5, 6, 7 s). Rats were considered to have acquired the task when they made greater than 60 correct responses and fewer than 20 errors of omission over 3 consecutive days. Upon reaching criteria, rats were challenged over 3 days by varying the stimulus duration (0.25, 0.5, 1, 1.25 s) within each session followed by 3 days on a schedule with multiple inter-trial intervals (1, 3, 5, 7 s). Performance measures included accuracy (correct/ correct + incorrect), number of premature responses, number of omissions, number of perseverative responses, latency to a correct response and latency to collect reward.

5C-CPT

Once a stable level of performance on the 5C-SRT was obtained, the rats were tested over fourteen days on the 5-choice continuous performance task (5C-CPT). This protocol consisted of 80 target and 40 non-target trials (total of 120 trials) per session [adapted from [26]]. The target trials were similar to those used during 5C-SRT with the following parameters; 1 s duration of the stimulus light, 5 s limited hold, a variable ITI (3, 4, 5, 6, 7 s), 5 s reward duration, and a 5 s time-out. During target trials responses were recorded as either a **hit** (correct response) or a **miss** (incorrect response + error of

omission). During the non-target trials the stimulus light appeared in all 5 apertures for 1 s and a response in any aperture resulted in a 5 s time out, during which the house light came on and was recorded as a **false alarm** (FA). Successful inhibition of a response during the 3-s limited hold resulted in a **correct rejection** (CR) and the delivery of a food pellet in the illuminated magazine. The non-target trials were pseudo-randomly interspersed within the 80 target trials, with a maximum of 3 sequential non-target trials. Response in any aperture during the ITI was recorded as a premature response and resulted in a 5 s time out.

In addition, the mean correct latency, mean premature latency, mean false alarm latency and percent omissions were recorded. The following parameters were also calculated, as described previously [26], hit rate, p(Hit) as the proportion of target trials correctly detected; false alarm rate, p(FA), as the proportion of non-target trials with an incorrect response; sensitivity index, SI, as a non-parametric measure of the ability to discriminate between target and non-target trials; and responsivity index, RI, as a non-parametric measure of response bias, and these are outlined in Table 1.

Treatment with Clozapine

DVD-deficient and control rats were then randomly divided to receive either an acute injection (i.p.) of either saline or clozapine (2.5 mg/kg, pH = 7.4, Sigma-Aldridge, St. Louis, MO) 1 h prior to testing on a single 5C-CPT session. This dose was carefully selected based on previous experiments [31,34-36].

Statistical Analyses

All data were analysed using the SPSS software package (ver. 17, SPSS Inc. IL, USA). Data obtained for the 5C-SRT and 5C-CPT were subjected to a repeated measures analysis of variance (ANOVA), with Maternal Diet as a between subjects factor and day as a within subjects factor. Significant differences were followed up with planned *post-hoc* bonferroni tests. Significance was set at $p < 0.05$. No significant interactions of Sex x Maternal Diet were observed and so all data were pooled for sex.

RESULTS

5C-SRT

DVD-deficient rats required more trials (21.5 $\pm$ 1.44) to reach criteria for level 7 (stimulus duration = 1 s) compared to controls (17.9 $\pm$ 1.34), although this difference did not reach significance ($F_{1,26} = 3.16$, $P = 0.087$). On the 3-day multiple stimulus durations level, rats were challenged to respond to a range of stimulus durations (0.2 – 1.0 s, Table 2). There was no significant effect of Maternal Diet on any measure of performance in the multiple stimulus durations task, including accuracy, omission rate, premature responses or perseverative responses ($P > 0.05$). At low stimulus durations, all rats made more omissions ($F_{3,78} = 11.81$, $P < 0.001$) and were less accurate when responding ($F_{3,78} = 127.56$, $P < 0.001$). On the 3-day multiple inter-trial intervals level (Table 3), there was no significant effect of Maternal Diet on performance over various inter-trial intervals on measures such as accuracy, omission rate, premature responses or perseverative responses ($P > 0.05$).

Over the different inter-trial intervals, the omission rate was fairly stable, except for an increase in omissions in trials after an inter-trial interval of 1.0 s, (Effect of inter-trial interval $F_{3,78} = 83.471$, $P < 0.001$).

5C-CPT

Over the 14-session 5C-CPT, DVD-deficient rats had similar levels of performance to controls on the primary measure of performance in target trials; p(Hit) (Fig. 1a). However, there was a significant effect of Maternal diet on the primary measure of performance in non-target trials; p(FA) ($F_{1,26}$ = 18.05.73, $P < 0.001$; Fig. 1b), and this was seen on the first session and persisted throughout all 14 5C-CPT sessions. With respect to signal detection DVD-deficient rats had significantly lower values for both RI ($F_{1,26} = 16.89$, $P < 0.001$) and SI ($F_{1,26} = 9.05$, $P < 0.01$) compared with control rats (Fig. 1c,d), and DVD-deficient rats also made more premature responses than control rats ($F_{1,26} = 4.25$, $P = 0.05$, Fig. 1e). There was no significant effect of Maternal diet on accuracy (Fig. 1f), omission rate or perseverative responses during the 5C-CPT sessions.

Treatment with Clozapine

Acute treatment of DVD-deficient rats with clozapine reversed the deficits in p(FA), reward latency and premature responses (Table 4). There was no significant main effect of Maternal diet on any measure after clozapine treatment ($P > 0.05$). Overall, acute treatment with clozapine in control and

DVD-deficient rats led to a significant increase in omissions and correct latency and a significant decrease in premature responses and p(Hit) ($F_{1,26}$ > 5.64, P < 0.025, for each comparison).

Saline-treated DVD-deficient rats had similar scores for p(Hit) but there was a significant effect of Maternal diet on p(FA) ($F_{1,26}$ = 18.10, P = 0.001, Fig. 2). DVD-deficient rats had a reduced reward latency ($F_{1,26}$ = 5.09, P = 0.044) and made more premature responses than control rats, although this failed to reach significance ($F_{1,26}$ = 3.15, P = 0.10). There was no significant effect of Maternal diet on omission rate, perseverative responses or correct latency after saline-treatment.

DISCUSSION

DVD-deficient rats demonstrated mildly enhanced impulsivity and while they were normal on all measures of vigilance on target trials, their lack of inhibition on non-target trials was observed immediately and persisted throughout testing. Controls showed a steady improvement in their ability to withhold responding over the 14 sessions of 5C-CPT testing; confirming Sprague-Dawley rats are capable of discriminating between target and non-target conditions. Maternal vitamin D deficiency decreased reward latency; suggesting motivational aspects of the task were also subtly altered. There was no effect of maternal diet detected under schedules of variable stimulus duration or inter-trial interval, indicating comparable stimulus discrimination and ability to attend when stimulus onset was unpredictable.

While premature responses on target trials did not differ between groups on the 5C-SRT, under the additional load of the 5C-CPT, DVD-deficient rats showed poorer inhibition of this erroneous behaviour. Interestingly, acute administration of clozapine resulted in reversal of these deficits in DVD-deficient rats.

The importance of extending the basic 5C-SRT to include the more challenging 5C-CPT component in animal studies is evident. Deficits were clearly and persistently detected once inhibitory demands were increased with the inclusion of non-target trials. The 5C-CPT protocol was designed to measure vigilance in mice and includes parameters of signal detection theory used during CPT testing in humans [26]. While the 5C-CPT protocol is a relatively new tool in cognitive testing, preliminary application to assess deficits associated with schizophrenia has found a reduction in behavioural inhibition in rats treated sub-chronically with PCP [37]. PCP administration has been used in a number of studies to model positive, negative and cognitive symptoms of schizophrenia [38-42]. These similarities suggest that the DVD deficiency rat model shares a cognitive deficit that has also been observed in another animal model of schizophrenia. Evidence that schizophrenic patients and first-degree relatives perform poorly on the human CPT strengthens the relevance of this tool to assess cognition.

Another behavioural change that only became apparent upon 5C-CPT testing was a significant decrease in the time taken for reward collection in DVD-deficient rats. Latency to reward collection was not found to differ in the 5C-

SRT, therefore it is unlikely that substantial motivational differences exist between control and DVD-deficient rats. However, the additional attentional load of the 5C-CPT appears to have altered motivational, impulsive and inhibitory behaviours. Given the relevance of motivational changes to schizophrenia, this alteration should be assessed separately under conditions designed to specifically measure motivation. The ability to assess and discriminate changes in complex cognitive behaviours is critical for modeling endophenotypes of mental disorders. Given the sensitivity of continuous performance tasks to detect differences in schizophrenia patients and rodent models, this protocol may be beneficial in the search for pro-cognitive therapeutics. The strongest predictive measure of favourable outcomes for schizophrenic patients have been found within neurocognitive domains and yet cognitive symptoms remain largely untreated by antipsychotic medications [5]. In light of this issue, further validation of the 5C-CPT to detect cognitive deficits in other animal models of schizophrenia and the effectiveness of novel drugs to reverse deficits is warranted.

Antipsychotic administration in this study found that acute clozapine treatment reduced false alarm responding in DVD-deficient rats. To the best of our knowledge, this is the first use of antipsychotics on the 5C-CPT. However, there have been numerous studies performed using antipsychotics in the 5C-SRT. In an animal model relevant to schizophrenia, deficits induced by PCP treatment were partially reversed by clozapine [31]. Repeated PCP injections were shown to decrease accuracy as well as increasing premature and timeout responding [31]. Chronic clozapine was able to improve accuracy and

reduce premature responding in these animals, while chronic clozapine alone did not alter accuracy or premature responding. This suggests clozapine is specifically acting to reverse PCP-induced attentional and inhibitory deficits. It is important to note that clozapine was only tested after acute administration in this study, however chronic use is required to provide relief to patients. It would be valuable to know if chronic administration could improve performance in a more stable manner, resulting in deficit reversal without drug administration immediately prior to testing. Although the current data are consistent with the finding that clozapine improves functioning in patients on attentional tasks [43], given the poor tolerability of clozapine in many patients, other compounds are needed to remediate cognitive deficits.

The action of clozapine is complex, interacting with a number of different receptor types including dopamine, serotonin and acetylcholine [44]. Clozapine has some affinity for the D2 receptor, however in the PFC clozapine has a higher affinity for 5-HT2a than for D2 receptors. The PFC is central to information processing, particularly when the correct response is not obvious and a decision between conflicting behaviours is required. Previous studies using the 5C-SRT have found that 5-HT2a blockade in the mPFC results in improvements in accuracy and anticipatory responses [45]. Within the PFC, inhibitory control of impulsivity may be selectively influenced by serotonin while perseverative responding may be guided by dopamine receptor systems [36]. These findings suggest a mode of action for clozapine in restoring the proportion of false alarms in DVD-deficient rats to control levels. It is also important to note that while omissions increased for both

control and DVD-deficient rats after clozapine, there was no effect of maternal diet indicating the improvement in inhibitory behaviour was not simply due to reduced responding.

This study has shown that the 5C-CPT is a useful tool in discriminating subtle differences, which may not be detected under less strenuous testing. Further validation of the use of antipsychotics, particularly clozapine, comparing acute and chronic administration to reverse inhibitory deficits on the 5C-CPT would also be encouraged. The DVD-deficient rat model is characterised by a phenotype reminiscent of both positive and negative symptoms of schizophrenia, and these results suggest that DVD-deficient rats also have specific impairments in cognitive function.

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Figure Legends

Fig. 1 Performance of control (○) and DVD-deficient rats (●) rats on the 5C-CPT. All rats had normal performance to target stimuli as measured by the probability of hit rate (a). DVD-deficient rats had increased probability of false alarm responding to non-signal stimuli (b) and they showed poorer vigilance as measured by the sensitivity index (SI, c), and a lower responsivity index (RI, d). Although DVD-deficient rats made more premature responses overall, compared to controls, this response was not stable. All rats maintained high levels of accuracy (f) throughout testing. Data presented as mean ± s.e.m. * $P < 0.05$, over all 14 sessions, repeated measures ANOVA.

Fig. 2 Performance of control (□) and DVD-deficient (■) rats on the 5C-CPT after treatment with clozapine, saline or at baseline. All rats had normal performance to target stimuli as measured by the probability of hit rate (a). DVD-deficient rats had increased probability of false alarm responding to non-signal stimuli at baseline and after saline treatment, but this was reduced by clozapine (b). DVD-deficient rats showed poorer vigilance as measured by the sensitivity index (SI, c), and a lower responsivity index (RI, d) at baseline and this effect was altered by clozapine. Saline treatment did not alter the SI but there was a significant reduction in RI from both control and DVD-deficient rats. Data presented as mean ± s.e.m. * $P < 0.05$.

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Figure 1
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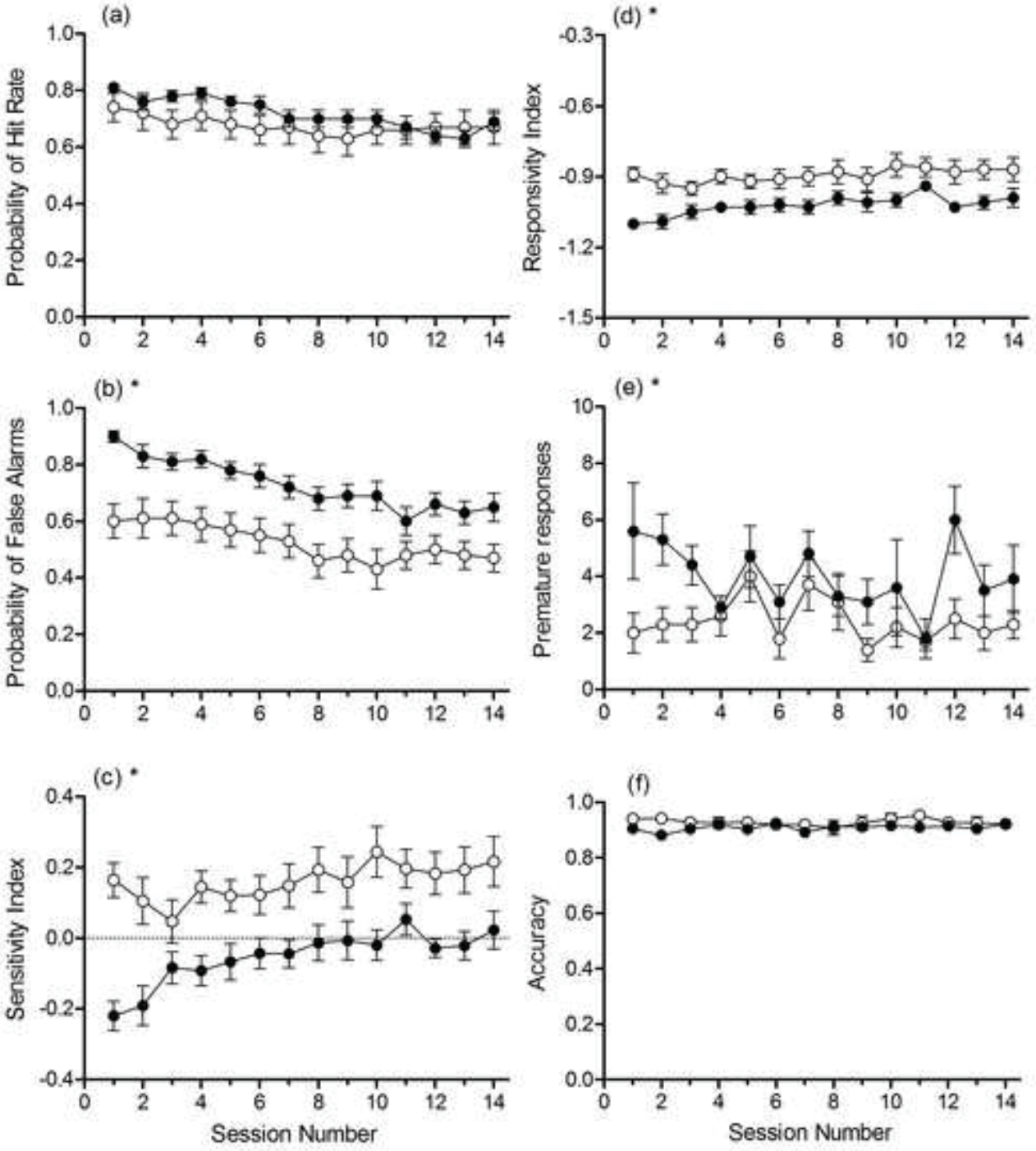

Figure 2

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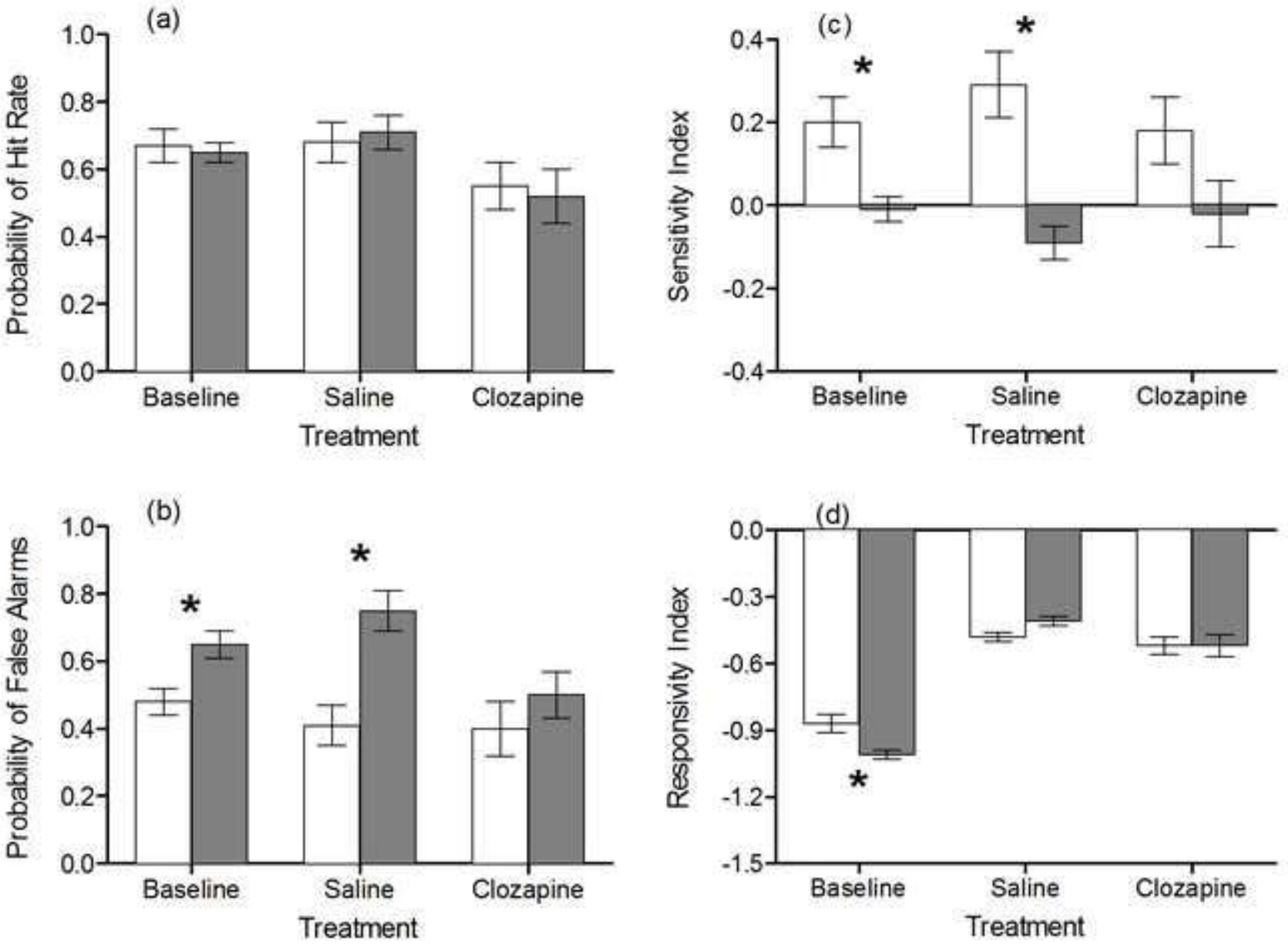

Table 1 Descriptions of the dependent variables used for the analysis of the 5C-SRT and 5C-CPT and how they were calculated.

Measure (Target Trials)	
Accuracy	(No. of correct responses) / (No. correct responses + no. incorrect responses)
p(Hit): Probability of a hit response	(No. of correct responses) / (Total no. of target trials)
Omission Rate	(No. of omissions) / (Total no. of target trials)
Premature Responses	No. of premature responses made
Perseverative Responses	A perseverative response occurred when a mouse nose-poked more than once in the correct hole
Latency to Correct Response (s)	Average time between target stimulus onset and correct nose poke
Latency to Retrieve Reward (s)	Average time between correct nose poke and reward retrieval
Measure (Non-target Trials)	
p(FA): Probability of a false alarm response	(No. of false alarm responses) / (Total no. of non-target trials)
Perseverative False Alarm	No. of perseverative false alarm responses made during non-target trials
Latency to False Alarm (s)	Average time between non-target stimulus onset and false alarm nose poke
SI: Sensitivity Index	$[p(Hit)-p(FA)] / (2[p(Hit)+p(FA)]-[p(Hit)+p(FA)]^2)$
RI: Responsivity Index	$[p(Hit)+p(FA)-1] / (1-[p(FA)-p(Hit)]^2)$

Table 2 Effects of DVD deficiency on 5C-SRT with a Shortened Stimulus Duration

		Stimulus duration (s)			
Measure	**Group**	**0.25**	**0.5**	**1.0**	**1.25**
(A) Accuracy (%)	CON	66.1 (2.1)	78.3 (2.1)	87.6 (2.3)	87.9 (1.6)
	DVD	63.4 (2.1)	79.4 (2.0)	86.6 (1.6)	87.9 (1.5)
(B) Premature (no.)	CON	14.9 (3.3)	15.5 (4.8)	15.6 (2.8)	14.6 (2.0)
	DVD	18.7 (2.8)	11.5 (1.8)	12.2 (2.2)	12.3 (1.5)
(C) Omission (no.)	CON	12.3 (1.1)	11.5 (1.5)	9.9 (1.0)	9.3 (1.4)
	DVD	15.1 (1.8)	12.6 (1.6)	10.8 (0.9)	9.0 (1.0)
(D) Perseverative (no.)	CON	3.2 (0.6)	5.3 (1.4)	6.2 (1.1)	5.5 (1.1)
	DVD	3.8 (0.8)	4.7 (0.9)	5.1 (0.8)	7.0 (1.4)
(E) Correct latency (s)	CON	0.57 (0.03)	0.62 (0.03)	0.66 (0.04)	0.65 (0.04)
	DVD	0.49 (0.04)	0.53 (0.04)	0.57 (0.04)	0.64 (0.03)
(F) Reward latency (s)	CON	1.49 (0.08)	1.47 (0.08)	1.45 (0.08)	1.45 (0.07)
	DVD	1.70 (0.06)	1.68 (0.06)	1.65 (0.06)	1.63 (0.06)

After the rats had acquired the 5C-SRT they were tested with a range of stimulus durations (0.25, 0.5, 1.0 and 1.25 s). The table shows mean (+ SEM) percent accuracy (A), premature responses (B), percentage of omitted trials (C), number of perseverative responses (D), latency to respond on trials with a correct response (E), and latency to collect reward (F) in DVD-deficient and control rats.

Table 3 Effects of DVD deficiency on 5C-SRT with Variable ITIs

		ITI Length (s)			
Measure	**Group**	**1**	**3**	**5**	**7**
(A) Accuracy (%)	CON	85.4 (2.9)	92.3 (1.4)	92.8 (1.2)	89.2 (1.1)
	DVD	85.5 (2.9)	89.9 (1.2)	93.5 (0.8)	88.0 (1.2)
(B) Premature (no.)	CON	15.8 (2.3)	6.6 (1.3)	11.2 (1.9)	27.7 (3.6)
	DVD	11.7 (1.8)	5.8 (1.0)	6.3 (0.8)	24.0 (3.3)
(C) Omission (no.)	CON	29.4 (3.7)	10.8 (1.6)	8.8 (1.5)	5.8 (0.8)
	DVD	31.9 (4.0)	14.9(2.4)	8.6 (1.0)	7.6 (1.3)
(D) Perseverative (no.)	CON	5.9 (1.4)	6.7 (1.2)	5.3 (1.3)	3.9 (1.5)
	DVD	7.2 (1.6)	6.8 (1.5)	5.5 (0.9)	4.1 (0.7)
(E) Correct latency (s)	CON	0.59 (0.05)	0.66 (0.02)	0.58 (0.03)	0.59 (0.03)
	DVD	0.51 (0.05)	0.57 (0.03)	0.59 (0.03)	0.54 (0.03)
(F) Reward latency (s)	CON	0.80 (0.07)	1.21 (0.08)	1.22 (0.06)	1.21 (0.06)
	DVD	0.88 (0.10)	1.22 (0.07)	1.39 (0.06)	1.31 (0.06)

After the rats had acquired the 5C-SRT they were tested with a range of ITIs (1, 3, 5 and 7 s). The table shows mean (+ SEM) percent accuracy (A), premature responses (B), percentage of omitted trials (C), number of perseverative responses (D), latency to respond on trials with a correct response (E), and latency to collect reward (F) in DVD-deficient and control rats.

Table 4 Effects of DVD deficiency on 5C-CPT under baseline conditions, or after treatment with saline or Clozapine

Measure	Group	Baseline	Saline	Clozapine
(A) Accuracy (%)	CON	92.9 (1.6)	84.0 (4.1)	84.3 (7.9)
	DVD	90.8 (0.6)	77.3 (2.8)	77.9 (5.7)
(B) Premature (no.)	CON	2.3 (0.5) *****	4.8 (1.3)	3.0 (1.3)
	DVD	4.5 (0.9)*	10.1 (2.4)	3.6 (1.6)
(C) Omission (no.)	CON	22.9 (4.0)	21.0 (4.9)	32.3 (5.4)
	DVD	22.9 (2.3)	16.4 (4.3)	30.0 (5.0)
(D) Perseverative (no.)	CON	3.3 (0.9)	6.5 (2.4)	3.0 (0.7)
	DVD	3.9 (0.7)	3.5 (1.1)	8.5 (3.2)
(E) Correct latency (s)	CON	0.83 (0.1)	0.78 (0.04)	0.89 (0.07)
	DVD	0.74 (0.1)	0.70 (0.05)	0.92 (0.09)
(F) Reward latency (s)	CON	2.54 (0.23)*****	2.76 (0.36)*	2.47 (0.21)
	DVD	2.08 (0.07)*	1.97 (0.15)*	2.65 (0.25)

The table shows mean (+ SEM) percent accuracy (A), premature responses (B), percentage of omitted trials (C), number of perseverative responses (D), latency to respond on trials with a correct response (E), and latency to collect reward (F), in DVD-deficient and control rats. * $p<0.05$ compared to Control value.