Modeling Symptoms of Attention-Deficit Hyperactivity Disorder in a Rat Model of Fetal Alcohol Syndrome

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Received 3 December 2015; Revised 11 January 2016; Accepted 10 March 2016

Abstract

Aims: Several studies indicate the similarity between the symptoms of fetal alcohol syndrome and attention-deficit hyperactivity disorder (ADHD). This study hypothesized that prenatal exposure to ethanol (EtOH) can be used as an animal model of ADHD in Wistar rats.

Methods: At the first stage of the study, alcohol was delivered to the pregnant dams (237–252 g) by intra-gastric route throughout Gestation Days 8–20 at a dose of 6 g/kg/day. Untreated control group with isocaloric sucrose intubation was also included. Of the 16 male pups (174–180 g), 8 were in the fetal alcohol effects (FAE) group and 8 were in the untreated control group. Subjects went through behavior shaping, discrimination learning and reversal learning. Number of sessions to learn the tasks, response frequency to inhibitory (S−) and excitatory (S+) stimulus features, response latency and inter-response time (IRT) were measured.

Results: Significant differences were obtained on only the reversal task. Rats with FAE needed greater number of sessions to learn the reversal task, and they had a higher frequency of incorrect responses in specifically the latter part of the sessions.

Conclusion: Our results suggest that reversal learning of FAE rats exhibits deficit in the inhibition of pre-learned responses. Responses behaviorally mimicked attention deficit and impulsivity symptoms of human ADHD. However, the experimental design of the study was not conducive to hyperactivity. Accordingly, rats with FEA can be an alternative to other models since it is not, for example, based on a symptom that is atypical (such as hypertension) to ADHD.

Short Summary: Significant difference was obtained in a reversal task between male rats prenatally exposed to ethanol and matched controls. The greater number of sessions for learning and higher frequency of incorrect responses behaviorally mimicked symptoms of ADHD, suggesting that rats with fetal ethanol effects can serve as a useful animal model.

INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is a diagnosis category defined by attention deficit, hyperactivity and impulsivity (American Psychiatric Association, 2013). ADHD is observed not only in childhood and adolescence but also in adulthood (Paris et al., 2015). The prevalence of ADHD in children is between 1.70 and 17.8% (Brown et al., 2001). In 78–85% of children diagnosed with ADHD, the disorder continues into adolescence, and for 50–70% of these children, it continues into adulthood (Lara et al., 2009).
In spite of its high prevalence, ADHD is still an unresolved medical problem (Davids et al., 2003; Sagvolden et al., 2005a). Focused experimental studies where different animal species serve as models can be conducted to unravel the ADHD mystery. For the model to be valid, symptoms should be induced by similar etiological mechanisms, and they should thus be reversed by similar psychopharmacological agents. The model should also allow predictions on behavioral, genetic and neurobiological aspects of the disorder (Davids et al., 2003; Sagvolden et al., 2005a,b).

Spontaneously hypertensive rat (SHR) model meets these validation criteria. However, SHR model has a limited value because it is based on a variable hypertension that is not specific to ADHD. When SHR rats are obtained in correlational designs, hypertensive rats are de facto aged (Sagvolden et al., 2005b). This is obviously inconvenient for studies on childhood ADHD. Accordingly, an optimal animal model for ADHD is still lacking.

Rodents prenatally exposed to ethanol (EtOH) show fetal alcohol effects (FAE). From childhood onwards (Sengupta, 2011), FAE animals behaviorally exhibit attention deficit (Leth-Stoengen et al., 2000; Hausknecht et al., 2005), hyperactivity and impulsivity (Gilbertson and Barron, 2005). Perinatal exposure to alcohol also leads to visuospatial deficits, another characteristic of ADHD (Reyes et al., 1989; Kalynchuk et al., 1997). Humans and rodents with such exposure show attention and learning impairments in place learning in the Morris Water Maze (Girard et al., 2000), spatial navigation in the radial arm maze (Reyes et al., 1989) and spontaneous alternation in the T-maze (Nagahara and Handa, 1997).

Animals prenatally exposed to EtOH show similarities to ADHD cases with regard to the affected brain areas. Neuronal reductions were reported in the medial prefrontal cortex (Mihalick et al., 2001), purkinje cells and cerebellar cortex (Dikranian et al., 2005). Abnormalities were also found at the hippocampal CA1 neurons and dentate gyrus (Jang et al., 2005). Functional studies found higher theta activity in specifically the CA1 area of hippocampus (Rubia, 2002).

ADHD is characterized with dopaminergic (DA) hypofunction at the mesolimbic, mesocortical and nigrostriatal DA pathways (Plezka, 2005; Sagvolden et al., 2005b). Animals with FAE also exhibit DA hypofunction (Wang et al., 2006). In humans, pharmacotherapy of ADHD is performed with agents that block DA transporters (e.g. methylphenidate and d-amphetamine). As in human ADHD, FAE in infra-human species can be reversed by central nervous system stimulants (Hamigan and Berman, 2000; Choong and Shen, 2004).

According to these findings, animals with FAE and humans with ADHD display clinical, neuroanatomical, neurophysiological and neurochemical similarities. Lately, these similarities formed the basis for even the clinical procedures. In this line, O’Malley and Nanson (2002) and O’Malley and Storoz (2003) report findings on the clinical and diagnostic implications and the therapeutic consequences of the link between FAE and ADHD.

Reversal learning, which is used for assessing acquisition and flexible adaptation to stimulus-reinforcement associations, is impaired in ADHD (Hart et al., 2013). Reversal performance specifically depends on the integrity of prefrontal cortical regions (Dias et al., 1996). This region is known to be impaired in ADHD (Itami and Uno, 2002). Consistent with this drug’s beneficial effects on cognitive processing in ADHD, high-impulsive individuals benefit from methylphenidate to a greater degree than low-impulsive ones (Clatworthy et al., 2009).

To be valid, an animal model has to mimic the behavioral, neuroanatomical and neuropsychopharmacological aspects of the disorder that it is being modeled. The aforementioned findings show that children with ADHD and animals with FAE show similarities in these three domains. According to the above behavioral, neural and neurotransmitter findings, animals with FAE can be used as a model of human ADHD to underpin the cognitive dynamics of the disorder. Thus, the aim of the present experimental study is to test the adequacy of the FAE model for mimicking the basic symptoms (attention deficit, impulsivity and hyperactivity) of human ADHD. It is hypothesized that rats prenatally exposed to alcohol would show symptoms of ADHD in reversal learning.

MATERIALS AND METHODS

Animals and laboratory conditions

This study was conducted on Wistar Albino rats. All procedures in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (USA) and the Declaration of Helsinki. Local ethical committee approval (No: 10/10 K-R) was also obtained.

Throughout the study, rats were housed in quiet, temperature- and humidity-controlled rooms (22 ± 2°C and 60 ± 5%, respectively). Lighting was maintained at a 12-hour light and 12-hour dark regime. Light-on period was between 07:00 and 19:00 hours; at other times, the room was kept dark. The study was performed in two stages: EtOH treatment stage, experimental stage. All experimental procedures were performed between 09:00 and 11:00 a.m.

EtOH treatment stage

In this stage, rat pups with FAE were bred from 30 females. Breeder rats were older than 70 days and weighed 237–252 g. Females rats were assigned to two groups by weight-matching: 20 were in the EtOH-injected (experimental group) and 10 were in the sucrose-injected (control) group. Of the 16 male rats, 8 were in the experimental group and 8 were in the control group.

Starting 1 week before fertilization, female rats were housed in Plexiglas cages in groups of three and were handled 15 minutes per day. Male rats were randomly assigned to female cages. Each day, male rats were placed in the female cages in the lights-off period (19:00–07:00 hours). The presence of vaginal plug was accepted as successful fertilization, and this day was marked as Gestation Day 1 (GD1). Pregnant rats were removed from the mating cages and were then individually housed.

This study used the technique of binge-drinking to produce FAE pregnant rats that were randomly assigned to the experimental and control groups. Rats in the experimental group were treated with EtOH via intubation. In order to control for a possible intubation-induced stress effect, rats in the control group were intubated with an identical volume of fluid which, however, contained sucrose isocaloric to ethanol. Intra-gastric intubation was performed using the standard stainless steel, curved feeding needles (18 gauges, 3 inches, Stoelting Co., USA) were used.

During the weekdays, daily doses (6 g/kg) of EtOH [20% (wt/vol)] or sucrose solution [30% (wt/vol)] were divided into two equal doses and delivered at 10:00 and at 15:00 hours. During the weekend, rats received a single dose of 4 g/kg EtOH. In line with the earlier studies (Hausknecht et al., 2005; Dursun et al., 2006), the doses were applied between GD 8 and GD 20. Blood ethanol concentration (BEC) was measured on GD 20, 1.5 hours after the second daily dose. GD 20 BEC was between 121 and 183 mg/100 ml, a value considered to be adequate for this kind of study.
Thiamine deficiency may produce adverse effects such as encephalopathy and neuroanatomical damage. Chronic alcohol consumption and the ensuing vitamin-B1 (thiamine) deficiency are associated with widespread neuroanatomical damage and learning deficits in rats (Ile and Markowitsch, 1983). To avoid the alcohol-induced thiamine deficiency and to control for its negative effect on cognition and task-related behavior, EtOH group was treated with thiamine (8 mg/kg, intramuscular) twice a week. In order to control for a possible injection-induced stress effect, the rats in the control group were injected with the same volume of saline.

After birth, male pups were reared and fostered by their natural mothers until postnatal (PN) Day 20. On PN 21, pups were weaned. Males were selected and housed in transparentplexiglass cages until 1 week before training. On PN 49, at approximately at the preadolescence stage (Sengupta, 2011), rats were weighed and examined for physical abnormalities. Weights were between 174 and 180 g.

Starting 5 days before training, rats were placed on a food-restricted schedule to attain approximately 80% of free-feeding weight. For this end, food was immediately restricted to one pellet (approximately 7.5 g) per day. The one pellet was in addition to those rats that could obtain through their operant responses.

**Experimental stage**

The second stage was the experimental study of reversal learning in the offspring of the EtOH and sucrose-treated breeders. Overall, 14 male rats were used in the study; 7 were in the prenatally EtOH exposed group and 7 were in the control group. One rat from each group was discarded due to physical problems.

Experiments on PN 55 rats were conducted in commercially available standard discrimination boxes (Coulbourn Instruments H-series, USA). The eight boxes were located in light- and sound-attenuated chambers. Each box contained a food magazine at the center of one wall, a pedal on the left side and three cue lights above the pedal. Pellets (45 mg, Bio-Serve, USA) were delivered through a programmable dispenser. Stimulus presentation and reinforcement delivery was controlled by a computerized automation system. Control and monitoring were performed using an IBM-compatible computer and Graphic State Notation version 2.000-00.

Reversal learning is a multistep procedure (Rubia, 2002). These steps are: (1) behavior shaping, (2) discrimination learning and (3) reversal learning. In behavior shaping, rats learned an operant response. They thus learned to get reinforcement (food pellets) by pressing the pedal. Learning criterion was a minimum of 30 pedal presses in a 20-minute session for a minimum of two consecutive sessions. After the criterion was reached, discrimination learning was initiated. In this step, rats learned to discriminate the functional stimulus. They learned that reinforcement is contingent on pressing the pedal in the presence of a discriminative stimulus (left led on: S+, excitatory stimulus). They learned that food would be available when they pressed the pedal in the presence of S+ and not present when they pressed the pedal in the presence of a second stimulus (right side led on: S−, inhibitory stimulus). The duration of the discriminative stimuli (S+ or S−) was 16 seconds and inter-stimulus interval was 1 second. S+ and S− stimuli were presented in a pseudorandom order according to a variable ratio schedule. Total number of S+ and S− stimuli was equal for each session. Learning criterion was a 70% response to the S+ stimulus during a 20-minute session for a minimum of two consecutive sessions.Criterion was reached in 10 sessions.

The first two steps were associated with operant learning, but the third step required response inhibition. After reaching the learning criterion for discrimination learning, reversal learning step was initiated. In reversal learning, reinforcement was contingent on reversing the pre-learned response. For this step, the valence of the discriminative stimuli was reversed such that S+ became S− and vice versa. As a result, what had been an excitatory stimulus in discrimination learning step became an inhibitory stimulus in the reversal learning step and what had been an inhibitory stimulus in the discrimination learning step became an inhibitory stimulus in the reversal learning step. In the reversal task, the criterion was again a 70% response to the S+ stimulus during a 20-minute session for a minimum of two consecutive sessions. Criterion was reached in 14 sessions.

**Experimental design and variables**

The study employed a factorial design. The factors (independent variables (IV)) in the experimental design changed with each step of the experiment. However, overall, factors were group (FAE group, control group), task (behavior shaping, discrimination learning, reversal learning), stimulus feature (S+, S−) and sessions (1–10 or 1–14).

The dependent variables of the experiment were as follows: (1) number of sessions necessary to reach the learning criterion, (2) response frequency: in discrimination and reversal learning tasks, correct response was pedal pressing in the presence of S+; incorrect response was pedal pressing in the presence of S−, (3) response latency; latency was the time difference (D) between stimulus onset (t1) and the first operant response to the stimulus (t2) (D = t2−t1), (4) average IRT: this was obtained by dividing total duration of a session (20 minutes/1200 sec) by the total response frequency in that session.

**Statistical analysis**

Analyses were performed using SPSS 13.00. Descriptive statistics included group mean and standard error of measurement. Multifactorial experimental design of the study was analyzed using analysis of variance (ANOVA). The number of factors (IV) and their levels changed according to the characteristics of each experimental step. When IV had more than two levels, the origins of significant differences were studied post hoc using Tukey test. The levels of statistical significance were set at P < 0.05.

**RESULTS**

**Dependent variable: Number of sessions**

Figure 1 illustrates the mean number of sessions in the FAE and control groups in each step of the experiment. A 2 × 3 ANOVA with repeated measures in the last factor included group (FAE, control) and task (behavior shaping, discrimination learning, reversal learning). Dependent variable was mean number of sessions required to reach the learning criterion.

Significant main effect was obtained for group [F(1,12) = 4.886, P = 0.047] Overall, mean number of sessions in the FAE group (11.85) was higher than that of the control group (10.38) (Fig. 1). Significant main effect was found for task [F(2,24) = 100.57, P < 0.0001]. Tukey test for the task effect revealed that in both the FAE and control groups, mean number of sessions were significantly higher (P = 0.05) in the reversal learning task (FAE: 19.43; control group: 15.14) than in the behavior shaping task (FAE: 5.43; control group: 4.86) and discrimination tasks (FAE: 10.71; control group: 11.14) (FAE: q2−12 = 8.72; q2−12 = 14.41 and q2−12 = 23.14, control group: q2−12 = 10.38; q2−12 = 6.61 and q2−12 = 16.99).

Interaction effect of group and task was significant [F(2,24) = 4.206, P = 0.027]. Tukey tests showed that mean number of
sessions in reversal learning task was significantly higher in the FAE group (19.43) when compared to the control group (15.14) \((q_{2-4} = 7.198, P < 0.01)\). Mean number of sessions in behavior shaping and discrimination learning tasks were not significantly different between the groups.

According to these findings, FAE group needed more sessions to reach criterion performance than the control group in only reversal learning. On behavior shaping and discrimination learning, groups were not significantly different. These results were, however, obtained in a more robust way when analyses were conducted on response frequency.

**Dependent variable: Response frequency**

**Discrimination learning**

Figure 2 illustrates the mean frequency of \(S^+\) and \(S^-\) responses in discrimination learning across FAE and control groups. Data on the discrimination learning task was analyzed using a \(2 \times 2 \times 10\) ANOVA with repeated measures in the last two factors. IV were group (FAE and control), stimulus feature (\(S^+\) and \(S^-\)) and sessions (1–10). Dependent variable was response frequency. Correct responses required responding to \(S^+\) stimuli (hit), incorrect responses (false alarm/false positive) required responding to \(S^-\) stimuli.

Significant main effect was found for stimulus feature \([F(1,12) = 21.739, P = 0.001]\). Overall, mean number of correct responses (30.96) was significantly higher than the mean of the incorrect responses (22.59) (Fig. 2). Significant main effect was found for session \([F(9,108) = 2.448, P = 0.014]\). Main effect of group was not significant.

There was a significant interaction effect between stimulus feature and session. Tukey test for stimulus and session interaction showed that the frequency of correct responses was significantly lower in the first part of the sessions \([\text{Session 2: Mean of correct (S+) responses (18.64) significantly lower than incorrect (S-) responses (32.14) } (q = 5.23; P < 0.05)\); Session 3: Mean of correct responses (24.36) significantly lower than mean of incorrect responses (39.21) \((q = 5.21; P < 0.01)\); Session 4: Mean of correct responses (25.21) significantly lower than mean of incorrect responses (39.00) \((q = 5.34; P < 0.01)\). Starting with the sixth session, however, stimulus feature effect became reversed. Frequency of correct responses became significantly higher than those for the incorrect responses \([\text{Session 6: Mean of the correct responses (30.93) significantly higher than mean of incorrect responses (19.21) } (q = 4.54; P < 0.01);\]

Session 7: Mean of correct responses (40.57) significantly higher than mean of incorrect responses (15.43) \((q = 9.74; P < 0.01)\); Session 8: Mean of correct responses (38.77) significantly higher than mean of incorrect responses (14.18) \((q = 9.53; P < 0.01)\); Session 9: Mean of correct responses (44.50) significantly higher than mean of incorrect responses (12.50) \((q = 12.40; P < 0.01)\); Session 10: Mean of correct responses (42.50) significantly higher than mean of incorrect responses (7.75) \((q = 13.47; P < 0.01)\). Other possible interactions, including the three-way interaction \([F(9,108) = 1.517, P = 0.151]\) were not significant.

According to these findings, FAE did not differentially affect discrimination learning neither with respect to the correct and incorrect response frequency nor with respect to the temporal response frequency layout over the sessions.

**Reversal Learning**

Figure 3 illustrates the mean frequency of \(S^+\) and \(S^-\) responses in reversal learning across FAE and control groups. Data on reversal learning task was analyzed using a \(2 \times 2 \times 14\) ANOVA with repeated measures in the last two factors. IV were group (FAE and control), stimulus feature (\(S^+\) and \(S^-\)) and sessions (1–14). Dependent variable was frequency of correct (\(S^+\)) and incorrect (\(S^-\)) responses.

Significant main effects were found for stimulus feature \([F(1,12) = 17.999, P = 0.001]\). Mean frequency of the correct responses (42.42) was lower than for the incorrect responses (58.77). There was a significant main effect of session \([F(13,156) = 2.164, P = 0.013]\). Mean response frequency at the 11th session was the highest (56.46), while the mean at the third session was the lowest (39.28). The main effect of group was not significant.

Significant effect was obtained for group \(\times\) stimulus feature interaction \([F(1,12) = 7.294, P = 0.019]\) (Fig. 3). Tukey test showed that the frequency of the correct responses of the FAE group (40.89) was lower than that of the control group (43.99) \((q_{1-12} = 6.948, P < 0.01)\); parallel to this finding, the frequency of the incorrect responses of the FAE group (67.65) was higher than that of the control group (49.89) \((q_{2-12} = 40.357, P < 0.01)\).

Significant effect was obtained for group \(\times\) session interaction \([F(13,156) = 1.861, P = 0.039]\) (Fig. 3). In approximately the first half of the experiment (Sessions 1–6), groups were not significantly different on response frequency. In approximately the second half of the sessions (7–14), response frequency of the control group steadily decreased while that of the FAE group was relatively steady.
At Session 8, the difference between the mean frequency of the total number of responses (to $S^+$ and $S^-$) of the two groups was significant at 0.05 level ($q = 3.70$); by Session 12, groups were significantly different at 0.01 level ($q = 5.27$).

Significant effect was obtained for stimulus feature × session interaction [$F_{(13,156)} = 8.35$, $P = 0.001$] (Fig. 3). Tukey test showed that the frequency of incorrect responses was significantly higher than correct responses until the last few sessions. At the 13th and 14th sessions, conditions reversed; correct responses become significantly higher than the incorrect responses ($q = 4.36, P < 0.01$) [Session 1: Mean of correct responses (22.36) significantly lower than mean of incorrect responses (83.00) ($q = 31.44$; $P < 0.01$); Session 2: Mean of correct responses (49.21) significantly lower than mean of incorrect responses (58.64) ($q = 4.88$; $P < 0.01$). Session 13: Mean of correct responses (49.57) significantly higher than mean of incorrect responses (41.14) ($q = 4.37$; $P < 0.01$). Session 14: Mean of correct responses (52.97) significantly higher than mean of the incorrect responses (40.27) ($q = 6.58$; $P < 0.01$). Other possible interactions, including the three-way interaction [$F_{(13,156)} = 1.413, P = 0.159$] were not significant.

According to these findings, response frequency of the FAE group is not significantly different from the control group when ANOVA’s main effect is considered. The situation with the FAE group is displayed in the interaction effects: (1) group × stimulus feature: The FAE group has a higher frequency of incorrect ($S^-$) responses; the control group has a higher frequency of correct ($S^+$) responses; (2) group × session: The groups are not significantly different for the first half of the sessions, while in the second half, response frequency of the control group is significantly lower than that of the control group; (3) stimulus feature × session: In the first part of the sessions, frequency of incorrect responses is significantly higher; in the last few sessions, the frequency of the correct responses is significantly higher. A scrutiny of Fig. 3 shows a spectacular situation. Groups do not in fact differ incorrect response frequencies. The incorrect response frequency of the FAE fluctuates around a high-level mean while that of the control group drastically decreases. The decrease in the incorrect response frequency of the control group gives way to the significantly higher correct response frequency.

**Dependent variable: Time**

A $2 \times 2$ ANOVA with repeated measure in the last factor included the following IV: group (FAE, control) and stimulus feature ($S^+$ and $S^-$). Dependent variable was response latency. None of the effects were found significant.

A $2 \times 2$ ANOVA with repeated measure in the last factor included the following IV: group (FAE, control) and task (discrimination learning, reversal learning). Dependent variable was average IRT. Main effect of task was found significant [$F_{(1,12)} = 120.47$; $P < 0.001$]. The average IRT for the discrimination task (26.35) was longer than for the reversal task (13.35) [$F_{(1,12)} = 120.47$; $P = 0.000$]. The difference between the average IRTs of the FAE and control groups was not significant.

**DISCUSSION**

As in the relevant literature (Hayward et al., 2004; BadanICH et al., 2011), this study did not find significant differences between the FAE and control groups in operant learning (behavior shaping and discrimination learning). Accordingly, FAE does not disrupt learning and/or response acquisition (Fig. 3). This conclusion applies not only to shaping and discrimination learning but also to reversal learning. FAE rats learn to commit and to make ‘hit’ responses to a previously unreinforced/inhibitory stimulus as readily as the rats in the control group.

In the reversal learning task, there is a change in the nature of the discriminative stimulus: subjects have to inhibit responding to the original $S^+$ stimulus (excitatory) and start responding to the originally inhibitory stimulus ($S^-$). When data are collapsed over the experimental conditions (main effect), frequency of responding was not significantly different between the FAE and control groups. Significant differences were obtained under specific combinations of conditions (interaction effect).

In the first part of the sessions, groups were not significantly different on incorrect responding (false alarm/false positive). However, in the latter part of the sessions, the frequency of incorrect responses decreased in the control group, while those in the FAE group kept on vacillating at a high level. Accordingly, in time, incorrect responses of FAE became significantly higher than that of the control group. These findings show that FAE disrupts learning to withhold or to inhibit responses.

According to basic science and clinical studies (Barkley, 1997; Bayliss and Roodenrys, 2000; Geurs et al., 2005), deficit in response inhibition is one of the basic underlying mechanisms of ADHD. The nature of this inhibition is explained in the literature in various ways. According to Sagvolden et al. (2005a); Sagvolden et al. (2005b), the causal factor of specifically the impulsivity symptom is the slower extinction of previously reinforced behavior. In the reversal task, subjects have to stop responding or to extinguish responding to the previously reinforced stimulus. Our study found that rats with FAE could reverse the previously reinforced operant response ($S^+$) in a greater number of sessions (Fig. 1). In line with the ‘slower extinction’ hypothesis of ADHD (Highfield et al., 1999), FAE rats could not extinguish a previously learned response as readily as the rats in the control group. A possible explanation of these findings is that rats with FAE display impulsive behavior.

However, reversal task has one extra step to extinction tasks. In reversal tasks, the subject not only needs to ‘withhold’ responding to a previously reinforced response but it also needs to learn and to ‘respond’ to a previously unreinforced stimulus (Rubia, 2002; Karakaş et al., 2013). Making such a response is contingent on effective interference control, and such a control represents the inhibitory aspect of at attentional modulation (Kane et al., 1994; Karakaş et al., 2013). The extra sessions needed for attaining

![Fig. 3. Frequency of correct and incorrect responses across groups (FAE and control) and sessions (1–14) in the reversal task. Rectangles: FAE group. Circles: control group. Open circles/rectangles: $S^+$. Closed circles/rectangles: $S^-$. *Significantly different from control groups.](image-url)
criterion performance and the greater number of incorrect responses (false alarm/impulsivity) show that FAE rats cannot effectively perform interference control. The occurrence of significant differences in the latter part of the sessions and the continuation of the false alarms over the session further show that the disturbance is stable over the studied time interval. A possible explanation of these findings is that rats with FAE display attention deficit.

According to a cognitive behaviorist approach to ADHD, the interval between the reinforcement and response, the delay gradient, is an important factor in ADHD (Sonuga-Barke et al., 1992; Sagvolden et al., 1993). According to this ‘delay aversion’ hypothesis, cases with ADHD prefer immediate reinforcers to delayed ones, even when the immediate reinforcer is of a lesser quantity. Accordingly, a rat conforming to the SHR model of ADHD does not exhibit hyperactive behavior when reinforcers are delivered frequently or in short intervals (Sagvolden et al., 1993). When we delivered reinforcement right after $S^+$ stimuli, we also did not find significant differences between the FAE and the control groups on either response latency or IRT. This finding is in line with the delay aversion hypothesis. However, such an experimental procedure put the study at a disadvantage when aim was to observe hyperactive behavior. The short time interval between operant response and reinforcement in our study did not allow for selective delay gradients and thus did not make hyperactive behavior, a distinctive symptom of ADHD, possible.

Another explanation of ADHD is the maturational lag hypothesis (El-Sayed et al., 2003). Studies on the neurodevelopmental aspect of ADHD present a spectrum of findings: Developmental retardation is observed in rats with FAE around 4 weeks of age; DA hypofunction of ventral tegmental area neurons appear at adulthood; FAE rats have reversal learning deficits on PN 28 which, however, wear out by PN 63; no impairment is observed in reversal learning when exposure to alcohol was at adolescence (Choong and Shen, 2004; O’Leary-Moore et al., 2006; McMurray et al., 2014). The variegated nature of these findings shows the necessity of conducting well-controlled studies on the subject. This study showed that there are differential changes in FAE and the control group throughout successive sessions of reversal learning. The experimental design of this study does not allow life-span developmental conclusions. However, it does allow monitorization of the temporal progression of behavior over the experimental sessions. The SHR model, for example, cannot serve as a useful model in development because these rats are de facto in the later stages of development (Sagvolden et al., 2005b). The experimental design and the tasks of this study can be useful in future longitudinal studies on the neurodevelopmental progression of ADHD symptoms.

A newly formulated concept attributes the cause of poor performance on reversal learning to ‘behavioral inflexibility’, defined as the impairment to modify responses when environmental demands change (Hamilton and Brigman, 2015). Authors suggest that impulsivity in ADHD and fetal alcohol syndrome, and also the other psychiatric disorders such as schizophrenia, can be described through behavioral inflexibility. Whether the symptoms of ADHD arise from inflexibility of an active inhibitory process or impulsive/perseverative behavior remains to be seen.

ADHD was once thought to be predominantly a male disorder. While this may be true for ADHD in childhood, extant research suggests that the number of women with ADHD may be nearly equal to that of men (Nussbaum, 2012). Accordingly, FAE may cause sexually dimorphic effects. This study was conducted on male rats. This choice was made primarily because males outweigh females in prevalence. In an epidemiological study, it was found that male prevalence of ADHD was 2.2% while female prevalence was 0.7% (Erskine et al., 2013). Furthermore, behavioral alterations that females display during the estrous cycle do not exist in the males.

The task of future research, where the experimental design and the procedures of this study are used, should be to investigate gender-specific responses to FAE.

We have some limitations in our study. First, drawing blood ethanol procedure was not performed on control animals. The act of drawing blood could be particularly stressful and introduces an unnecessary confound into the study. Second, the experimental design of this study did not manipulate response-reinforcement interval as a result of which hyperactivity was not obtained. Future studies should also add response-reinforcement interval to the experimental design of this study. Such an approach would allow the study of FAE on hyperactivity. Then, the test of the ‘delay aversion’ hypothesis of ADHD would also become possible. In future studies, blocking dopamine reuptake using methylphenidate or d-amphetamine and then rescuing deficits in reversal learning in the operant conditioning task used in this study support our hypothesis considerably. In addition, combining fetal FAE with some other sort of manipulation that results in hyperactivity is helpful to create a much more valid model for ADHD. In addition, the finding about the extra sessions needed for attaining criterion performance and the greater number of incorrect responses (false alarm/impulsivity) led us to conclude that FAE rats cannot effectively perform interference control. This conclusion should be tested by controlling the possible contaminating effects of memory and motivation.

In conclusion, FAE rats confronted with a reversal learning task behaviorally, which mimic attention deficit and impulsivity symptoms of human ADHD. Hyperactivity was not obtained due to the experimental design of the study. According to these findings, rats with FEA can be an alternative to SHR model, especially since it is not based on a symptom (such as hypertension) that is atypical to ADHD.

**FUNDING**

This research was conducted as a requirement of the M.S. Program of the Institute of Social Sciences of Hacettepe University, Turkey in the area of in Experimental Psychology. The study was partially supported by Scientific and Technological Research Council of Turkey (TUBITAK) (Project No: SBAG-110S344).

**CONFLICT OF INTEREST STATEMENT**

None declared.

**ACKNOWLEDGEMENTS**

The authors would like to thank Dr Teyfik Alci and Dr Hakan Kayir for their valuable contribution to behavioral measurements, and to Mr Selami Alan for technical assistance in data acquisition.

**REFERENCES**


