

Behavioral Biomarkers of Schizophrenia in High Drinker Rats: A Potential Endophenotype of Compulsive Neuropsychiatric Disorders

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Psychogenic polydipsia, which is compulsive, non-regulatory fluid consumption, is present in 6%–20% of chronic psychiatric patients and frequently associated with the schizophrenia diagnosis. In the present study, we investigated the relation between schizophrenia-like symptoms and biomarkers with a compulsive drinking behavior phenotype in rats. Rats that were selected for low drinking vs high drinking behavior following schedule-induced polydipsia (SIP) were assessed in a latent inhibition (LI) paradigm using tone and electrical foot shock and in a spatial reversal learning task to evaluate behavioral inflexibility. We also analyzed the myelin basic protein in different brain areas of high drinker (HD) and low drinker (LD) rats. The HD rats, which were characterized by a compulsive drinking behavior on SIP, had a reduced level of LI effect and increased behavioral inflexibility in the spatial reversal learning task in comparison to the LD group. Moreover, HD rats showed less myelination in the center of the corpus callosum, striatum, and amygdala in comparison to LD rats. These findings strengthen the validity of HD rats that were selected by SIP as a possible phenotype of compulsive neuropsychiatric disorders, as evidenced by the existence of behaviors and biological markers that are related to schizophrenia and obsessive-compulsive disorder, including a reduced LI effect, behavioral inflexibility and reduced brain myelination. Future studies could contribute to the elucidation of the mechanisms underlying the compulsive phenotype of HD rats and its relation to vulnerability to schizophrenia.

Key words: compulsivity/schizophrenia/
schedule-induced polydipsia/reversal learning/latent inhibition/myelination

Introduction

Schizophrenia is a highly debilitating neuropsychiatric disorder that affects 1% of the world population.¹ Psychotic symptoms affect almost all aspects of mental activity, including perception, attention, memory, and emotion.² One of the core symptoms that is observed in schizophrenia is engaging in repetitive thoughts and behaviors, which fosters a comorbidity with other neuropsychiatric disorders that are characterized by compulsive behaviors, such as obsessive-compulsive disorder (OCD) or autism.^{3,4} Moreover, schizophrenic patients are also characterized by behavioral signs, such as the attenuation or absence of latent inhibition (LI),^{5,6} which is a neural mechanism that allows for irrelevant stimuli to be ignored,⁷ and behavioral inflexibility, which is defined as the lack of ability to adjust and respond to changes in the environment.^{8,9}

Currently, the antipsychotic medication has shown a reduced efficacy for the treatment of neurocognitive and social dysfunction symptoms.¹⁰ A proposed underlying neurobiological mechanism includes myelin abnormalities in the corpus callosum, which is hypothesized to be a central biomarker of the disorder.^{11–13} Moreover, OCD patients have also shown myelin abnormalities, in terms of white matter volume and microstructure, primarily located within the fronto-striato-thalamo-cortical circuit.^{14–16} Thus, the relationship between compulsivity and white matter abnormalities appear to be important behavioral and biological markers of vulnerability to compulsive spectrum disorders.

The animal model of scheduled-induced polydipsia (SIP), which is defined as excessive drinking behavior in food-deprived animals that is not result of physiological needs but rather a response to intermittent reinforcing

program,^{17,18} has been shown to be a useful tool for exploring the neurobiology of compulsivity in different neuropsychiatric disorders.^{19,20} These include schizophrenia,^{21–23} OCD,^{24–27} and alcohol abuse.^{28–31} Indeed, psychogenic polydipsia (PP), which is defined as excessive non-physiologic drinking, is present in 6%–20% of psychiatric patients with schizophrenia and other compulsive spectrum disorders.^{32–34}

SIP is a model that is sensitive to individual differences, where rats can be selected according to their compulsive drinking pattern.^{20,35,36} Previous studies that were performed in our laboratory have shown relevant differences between high drinker (HD) and low drinker (LD) rats that were selected according to SIP acquisition. For example, the behavioral response to a challenge with dopaminergic drugs, such as D-amphetamine and cocaine,³⁷ diverges in the availability of dopamine D₁ and D₂ receptors in mesolimbic brain areas³⁸; lack of inhibitory control in the 5-choice serial reaction time task (5-CSRTT); increased serotonin and noradrenalin activity in the amygdala in post-mortem brain analyses³⁹; and a reduction of compulsive drinking by the 5-HT_{2A/C} serotonin agonist DOI in HD rats.⁴⁰ HD rats selected by SIP have shown behavioral and neurochemical alterations that are characteristic of inhibitory control deficits; however, the knowledge of the possible co-occurrence with other behavioral biomarkers of schizophrenia remains unknown.

In the present study, we investigated if the compulsive rats, HD selected by SIP, showed possible differences, compared with LD rats, in behavioral and biological markers of schizophrenia. The HD rats showed: a reduced effect in a LI paradigm, considered as a behavioral model of schizophrenia^{7,41–43}; an increased behavioral inflexibility in a spatial reversal learning task,^{44,45} compared with LD rats. Finally, according the brain myelin abnormalities found in schizophrenia and OCD patients, we assessed the myelin basic protein (MBP) staining in the corpus callosum, striatum and basolateral amygdala. Our results showed a reduced MBP staining in HD compared with LD rats.

Methods

Subjects

Sixty-one male Wistar rats (Harlan Ibérica), weighing approximately 275–300 g at the start of the experiments, were housed 3 per cage (50 × 15 × 25 cm) at 22°C and under a 12-hour light–dark cycle (light off at 08:00 AM), with food and water available ad libitum. After 10 days of habituation, rats were gradually reduced to 85% of their free-feeding body weight by controlled feeding and maintained at this level of deprivation throughout the experiment. Food was available by daily feedings of lab chow approximately 30 minutes after each experimental session. All testings were conducted between 09:00 AM and 02:00 PM. All procedures were performed in accordance with Spanish RD1201/2005 on the protection of experimental animals and the European Community Council Directives (86/609/EEC).

Experimental Procedures

Schedule-Induced Polydipsia. Two cohorts of rats (cohort 1: $N = 32$ rats and cohort 2: $N = 29$ rats) were tested in 13 operant SIP chambers (32 × 25 × 34 cm) (MED Associates). After 20 daily sessions of SIP, the animals were separated into 2 specific populations, HD and LD, according to whether their rates of drinking were above or below the group median, respectively.^{20,40} The following measures were recorded for each rat: (a) total amount of water (milliliters) removed from the bottle, (b) total licks to the bottle and (c) total entries into the food magazine ([supplementary methods](#)).

The order of screening and testing was the following: for the first cohort, SIP and LI were tested; for the second cohort, SIP and the Reversal Learning task were tested.

Latent Inhibition. After the separation of HD ($n = 16$) and LD ($n = 16$) rats by the SIP procedure in the first cohort, selective attention was tested by a LI paradigm. Animals were formerly trained in the lever press. Then, 1 group was pre-exposed to a tone, and finally, all animals were tested in conditioned suppression of the lever press.^{46,47} The variable measured was the suppression ratio ([supplementary methods](#)).⁴⁶

Reversal Learning. After the SIP procedure, the second cohort of HD ($n = 14$) and LD ($n = 15$) rats was tested for behavioral inflexibility by the Spatial Reversal Learning task. The animals were required to learn the initial spatial location of a reinforced lever and to then press the correct lever when the position was reversed. The task procedure was based on Boulougouris et al.⁴⁸ and previous studies.^{45,49,50} The variables measured were: the number of trials to reach the criterion, number of incorrect responses, perseverative errors, learning errors, latency to collect the food pellet, latency to respond to levers, and omissions ([supplementary methods](#)).

MBP Immunohistochemistry. After termination of behavioral experiments, 8 non-pre-exposed rats from the HD ($n = 4$) and LD ($n = 4$) groups of the first cohort (LI) were used to study myelination according to the method of Fuentes et al.⁵¹ Different brain regions were selected according to the rat brain atlas of Paxinos and Watson ([supplementary methods](#)).⁵² The results of the measure of each animal are expressed as the average density of both brain hemispheres.

Statistical Analyses

Data were analyzed using ANOVA or Student's *t* test when appropriate ([supplementary methods](#)). Data in all figures are shown as the mean ± standard error of the mean (SEM). Statistical significance was set at $P < .05$.

Results

HD and LD Selected by SIP

The mean of water intake, licks and magazine entries of HD and LD rats on SIP are shown in [figure 1](#) and [supplementary table S1](#). In the 2 cohorts, HD rats increased water intake (interaction SIP session \times group effects: group 1 $F_{19,570} = 18.16$, $P < .0001$; group 2 $F_{19,513} = 9.29$, $P < .0001$) and licks (interaction SIP session \times group effect: cohort 1 $F_{19,570} = 6.83$, $P < .0001$; cohort 2 $F_{19,513} = 14.03$, $P < .0001$) across SIP sessions compared with the LD group ([figures 1A](#) and [1B](#)). Post hoc analysis indicated a significant increase in water intake on SIP of HD in comparison to LD rats starting at session 3 in cohort 1 ($P < .01$) and at session 5 in cohort 2 ($P < .0001$). Furthermore, compared to the first session, HD animals significantly increased their consumption of water during session 3 for cohort 1 ($P < .05$) and during session 4 in cohort 2 ($P < .01$), reaching stable levels of water intake at session 11 in cohort 1 and at session 10 in cohort 2. In addition, LD animals did not show a significant increase

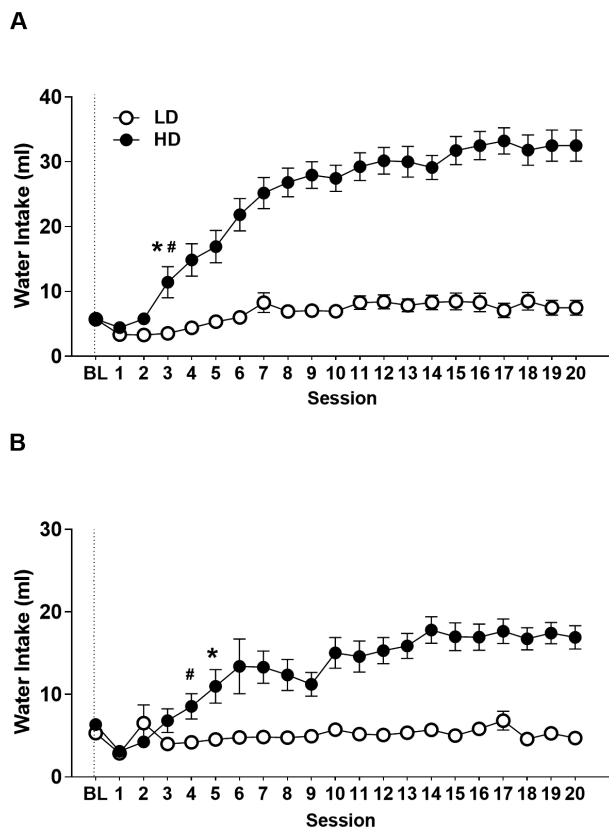


Fig. 1. Schedule-induced Polydipsia, the mean (\pm SEM) water intake in FT-60s across 20 sessions of high drinker (HD) and low drinker (LD) groups, cohort 1 in panel A (HD, $n = 16$; LD, $n = 16$) and cohort 2 in panel B (HD, $n = 14$; LD, $n = 15$). * $P < .05$ indicates significant differences between HD and LD groups from that session onward. # $P < .05$ indicates significant differences in comparison to session 1 on schedule-induced polydipsia (SIP) in the same group.

in their consumption of water across SIP sessions. In the number of total licks, HD rats showed a significant increase in comparison to LD rats that started during session 2 in cohort 1 ($P < .01$) and during session 1 in cohort 2 ($P < .01$). There were no significant differences between the HD and LD rats in the magazine entries measured on SIP ([supplementary table S1](#)).

Latent Inhibition

Training Sessions. The means of lever pressing for HD and LD rats in the VI30 s and VI60 s reinforcement schedules are shown in [supplementary figure S1](#). In the VI60 s schedule, HD rats revealed a significant increase in lever pressing in comparison with LD rats ($t(30) = -2.92$; $P < .05$). No differences were observed in VI30 between HD and LD rats ($t(30) = -1.15$, $P = .25$).

Conditioning. The mean of suppression ratios of HD and LD rats in the LI paradigm are shown in [figure 2](#). In the LD group ([figure 2A](#)), the pre-exposure affected the conditioning phase, as shown by an increased suppression ratio response (interaction pre-exposure \times session: $F_{3,42} = 2.88$, $P < .05$; pre-exposure effect: $F_{1,14} = 18.12$, $P < .001$; session effect: $F_{3,42} = 6.63$, $P < .001$). Post hoc analysis indicated that PRE rats had an increased response in comparison to no-PRE rats from session 1 to 3 ($P < .001$), while they displayed a trend towards still maintaining significant differences at session 4 ($P = .056$). In the HD group ([figure 2B](#)), the pre-exposure affected the conditioning phase, as shown by an increased suppression ratio response (interaction pre-exposure \times session: $F_{3,42} = 4.72$, $P < .001$; session effect: $F_{3,42} = 19.56$, $P < .0001$; no pre-exposure effect: $F_{1,14} = 1.86$, $P = .19$). However, post hoc analysis revealed that there was a reduced pre-exposure effect in HD rats that showed differences in the suppression ratio response of PRE rats in comparison to no-PRE rats only at sessions 1 and 2 ($P < .05$, $P < .01$), but not at sessions 3 and 4 ($P = .73$, $P = .90$), during the conditioning phase. [Figure 2C](#) shows the different performance by the mean of the suppression ratio for session 3 and 4 under the pre-exposure ($t(14) = 2.28$, $P < .05$) vs the no pre-exposure condition ($P > .05$) in the HD and LD rats.

Reversal Learning

Trials to Criterion. The mean of the number of trials to reach the criterion of the Reversal Learning task are shown in [figures 3A](#) and [3B](#). During retention phase, HD rats had an increased in number of trials to complete the criterion in comparison to LD rats (interaction group \times retention: $F_{3,81} = 5.31$, $P < .05$; group effect: $F_{1,27} = 5.45$, $P < .05$; retention phase effect: $F_{3,81} = 9.63$, $P < .0001$). Post hoc analysis revealed that there were no differences in the spatial discrimination retention between HD and LD rats, however, HD rats had a significant increase in

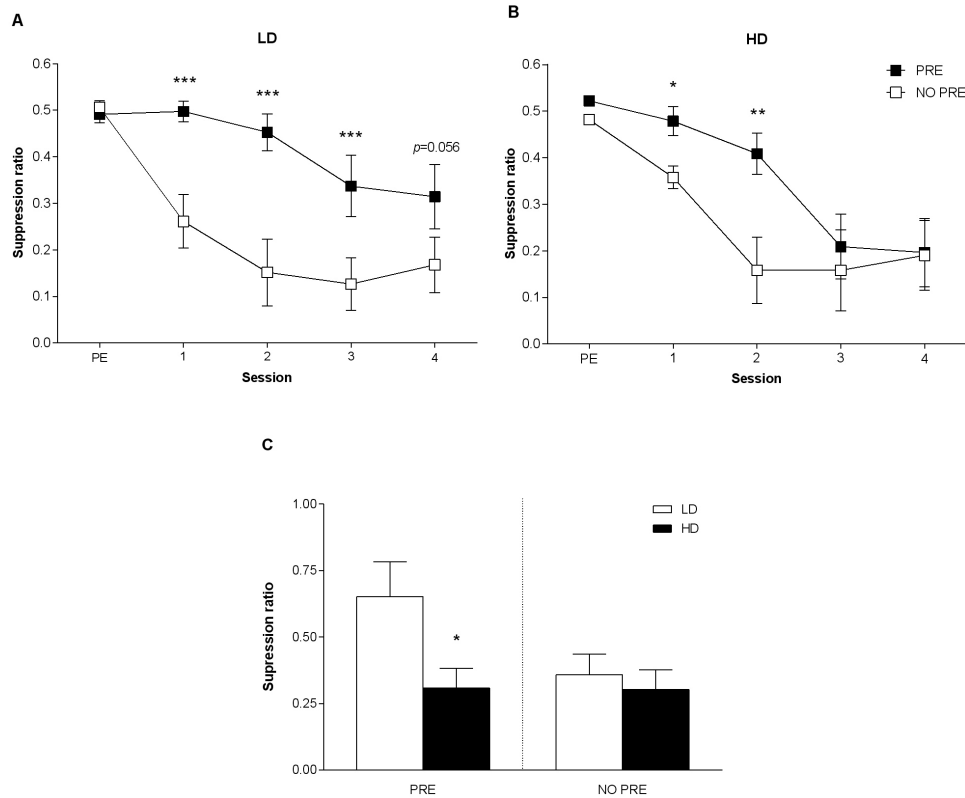


Fig. 2. Latent inhibition, suppression ratio for the last day of pre-exposure and the 4 sessions of conditioning in the low drinker (LD) group in panel A (LD NO-PRE $n = 8$; LD PRE $n = 8$) and high drinker (HD) group in panel B (HD NO-PRE, $n = 8$; HD PRE, $n = 8$). Panel C, suppression ratio mean for the 2 last conditioning sessions (3 and 4). Data are expressed as the mean \pm SEM. In panel A and B, $*P < .05$, $**P < .01$ and $***P < .001$ indicate significant differences between the pre-exposed and not pre-exposed groups. In panel C, $*P < .05$ indicate significant differences between HD and LD groups.

the number of trials to reach the criterion in the retention of reversal 1 in comparison to the LD group ($P < .0001$). These differences did not remain during the retention of reversals 2 and 3 (figure 3A). During reversal, HD rats needed an increased number of trials to reach the criterion in comparison with LD rats (interaction group \times reversal: $F_{3,81} = 3.81$, $P < .01$; group effect: $F_{3,81} = 30.65$, $P < .0001$). Post hoc analysis revealed that HD rats needed significantly more trials to reach the criterion during reversal 1 ($P < .01$) and also during reversal 2, with a trend towards a significant difference ($P = .09$) in comparison to LD rats (figure 3B).

Incorrect Responses to Criterion. The mean of the incorrect responses to the criterion on the Reversal Learning task are shown in figures 3C and 3D. In the retention phase, HD rats had an increase in incorrect responses (interaction group \times retention: $F_{3,81} = 2.96$, $P < .05$; retention effect: $F_{3,81} = 4.55$, $P < .01$). Post hoc analysis of retention phase revealed no significant differences in spatial discrimination retention between HD and LD rats, however indicated that HD rats made significantly more incorrect responses during retention of reversal 1 than LD rats ($P < .01$) (figure 3C). During the reversal phase, HD rats needed an increased number of incorrect

responses to reach the criterion (interaction group \times reversal: $F_{3,81} = 2.91$, $P < .05$; session effect: $F_{3,81} = 56.85$, $P < .0001$; no group effect $F_{1,27} = 2.91$, $P = .14$). Post hoc analysis revealed that HD rats made significantly more incorrect responses during reversal 1 ($P < .01$) and also during reversal 2, with a trend towards a significant difference ($P = .06$) in comparison to LD rats (figure 3D).

Perseverative and Learning Errors. The mean of perseverative and learning errors in the reversal learning task are shown in figures 4A and 4B. During reversals, HD rats showed a significantly increased number of perseverative errors in comparison to LD rats (interaction group \times session effect: $F_{3,78} = 2.55$, $P < .01$; session effect: $F_{3,78} = 55.26$, $P < .001$; no group effect $F_{1,26} = 1.82$, $P = .18$). Post hoc analyses revealed that HD rats had a significantly increased number of perseverative errors during reversal 1 ($P < .01$) in comparison to LD rats (figure 4A). In contrast, during reversal, no significant differences were observed in learning errors measure (figure 4B).

Omissions and Latencies to Respond. No significant differences were found between HD and LD rats in either omission (supplementary table S2) or in latency

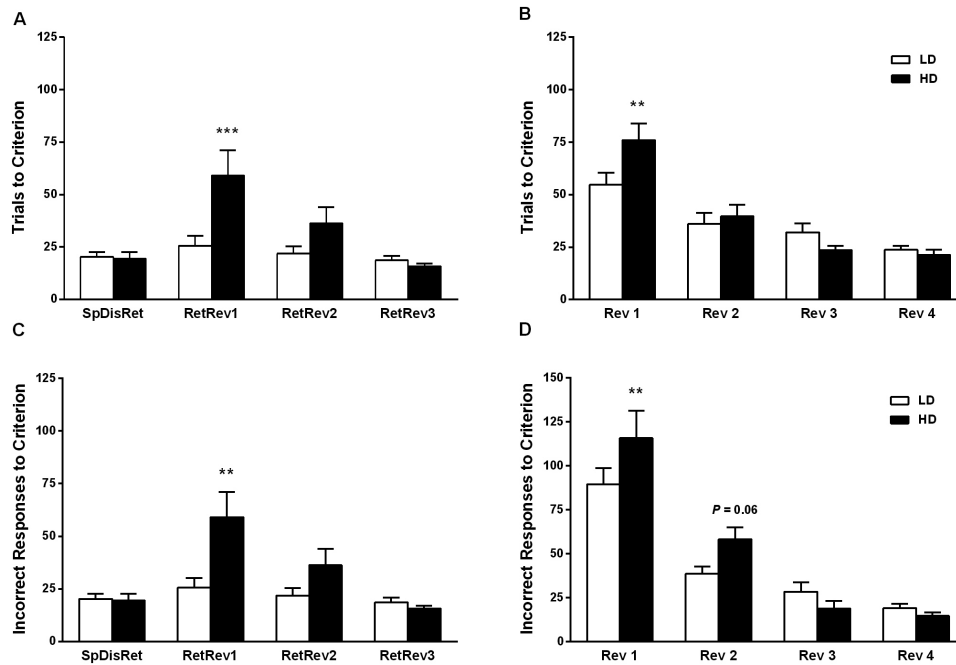


Fig. 3. Reversal learning task, number of trials to criterion and incorrect responses to criterion through (A, C) the retention phase as well as the (B, D) reversal phase of the experiment. Abbreviations, SpDisRet: Spatial Discrimination Retention; RetRev1: retention reversal 1; RetRev2: retention reversal 2; RetRev3 Ret: retention reversal 3. Data are expressed as the mean \pm SEM. ** $P < .01$ and *** $P < .001$ indicates significant differences between the high drinker (HD; $n = 14$) and low drinker (LD; $n = 15$) rats.

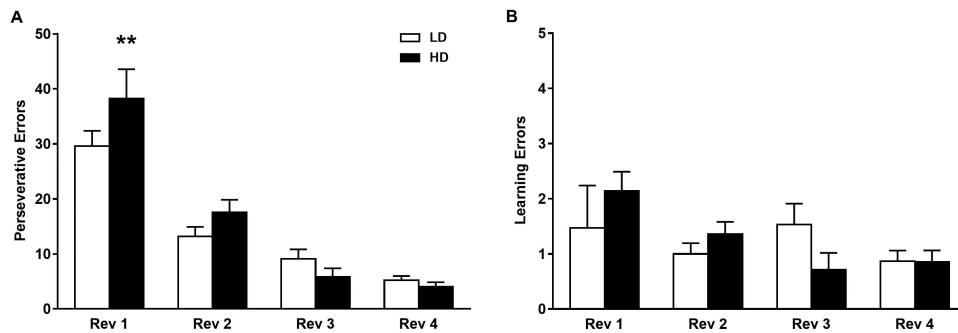


Fig. 4. Reversal learning task, mean error scores \pm SEM of the high drinker (HD) and low drinker (LD) groups: (A) perseveration (≤ 6 consecutive presses on the wrong lever) and (B) learning (> 6 lever presses on the wrong lever). ** $P < .01$ indicates significant differences in the number of perseverative errors between the HD and LD rats.

to response (supplementary table S3) at any stage of the experiment.

Myelination in HD and LD Rats

The analyses of MBP revealed significant differences in the optical densities of myelinated fibers between HD and LD rats (Figure 5). HD rats showed reduced myelination within the corpus callosum ($t(35) = -3.73$, $P < .001$), striatum ($t(15) = -2.51$, $P < .05$) and basolateral amygdala ($t(62) = -3.44$, $P < .001$) in comparison to LD rats.

Discussion

In the present study, we demonstrated that an animal model of compulsivity, HD rats selected by SIP, showed

the existence of other behaviors and biological markers that were related to schizophrenia and OCD. The HD rats, which were characterized by a compulsive drinking behavior on SIP, showed less LI effect, as indicated by a reduced effect of pre-exposure to stimuli in a conditioned emotional response task; and increased behavioral inflexibility in a spatial reversal learning task in comparison to LD rats. Moreover, HD rats showed less myelination in the center of the corpus callosum, striatum, and basolateral amygdala compared with LD rats.

Latent Inhibition

During training previous to the pre-exposure phase, we found that HD compulsive rats selected by SIP showed significantly increased lever presses than LD rats under

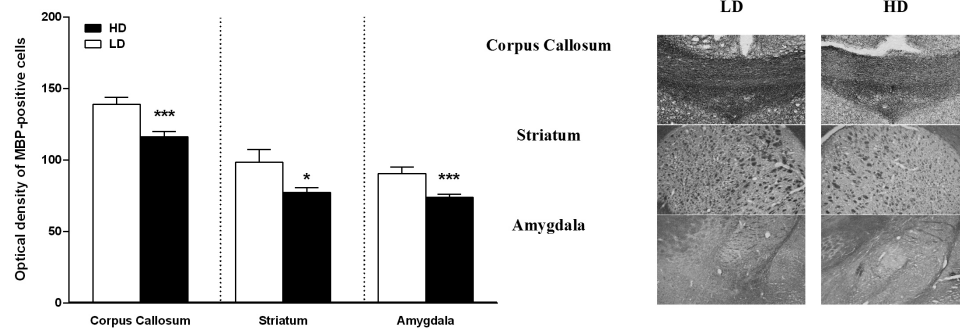


Fig. 5. Expression of myelin basic protein (MBP) in the corpus callosum, striatum, and basolateral amygdala in the high drinker (HD; $n = 4$) and low drinker (LD; $n = 4$) rats selected by schedule-induced polydipsia (SIP). Data are expressed as the mean \pm SEM. * $P < .05$ and *** $P < .001$ indicate significant differences between the HD and LD rats.

the VI60 s schedule. Previous studies have posited the idea that training under intervals may induce habit formation responses. Lever pressing is a response that is used in different animal models of compulsivity,^{53,54} such as the reward omission procedure^{55,56} and the post-training signal attenuation model (PTSA).^{57,58} Our results in the VI60 s schedule are coherent with the above-cited studies, because HD rats continue pressing without earning additional pellets. Future studies based in the stimulus-response habit formation should explore if HD rats might be a possible sensitive population.

HD rats had a reduced LI effect in comparison with LD rats. In HD rats, the pre-exposed group only showed differences during session 1 and 2 compared with the pre-exposed group, while in the LD group, the LI effect was maintained until session 4. The disruption of the LI effect through different procedures has been used as a model of schizophrenia.⁴³ Lesion studies in rats have demonstrated the implication of different brain structures in this phenomenon: the nucleus accumbens shell,^{59,60} basolateral amygdala, entorhinal cortex,^{61,62} and ventral hippocampus.⁶³ Moreover, the pharmacological manipulation of NMDA receptors, which is as an animal model of schizophrenia, also induced LI disruption.⁶⁴ Likewise, recent experiments have shown that the administration of a NMDA receptor antagonist, MK-801, in rats increased water intake on SIP.²¹ The idea that a compulsive trait could also be accompanied with an abnormal LI has also been observed in human studies with contradictory results. Two studies found enhanced LI in OCD patients,^{65,66} whereas other studies have shown deficits in LI depending upon the OCD subtype.⁶⁷

Reversal Learning

The HD compulsive rats that were selected by SIP, showed behavioral inflexibility, as reflected by their significant poorer performance in all measures of the spatial reversal learning task, in comparison to LD rats. Thus, during reversal 1, HD rats needed more trials to reach the criterion as well as had more incorrect and perseverative

responses than LD rats. In terms of behavioral inflexibility, the failure in the trials to criterion, although perseverative in nature, reveals that HD rats might have a deficit in the initial reversal learning phase, thus in the detection of the contingency changes. This deficit was also accompanied by a lack of the inhibitory response revealed by the increased incorrect perseverative responses. In contrast, there were no differences in the number of learning errors or the acquisition and retention of the spatial discrimination between HD and LD rats, which supports the notion that the differences were not due to a deficit in the acquisition or retention of previous stimulus reward contingencies. Moreover, the finding that the significant differences are only found during the reversal 1 are in accordance with previous literature^{45,48-50}, and might be explained by the large number of trials to criterion done in this reversal, that could benefit the performance in the next reversals. The relation between compulsive behaviors, thus behavioral inflexibility and repetitive drinking behavior on SIP, could be established by comparing several studies. First, animal lesion studies have previously demonstrated the implication of the orbitofrontal cortex (OFC) in impaired reversal learning.^{44,48,68} Moreover, in OCD patients and their clinically unaffected relatives, there is an abnormally reduced activation of OFC during reversal learning.⁶⁹ According to these results, recent c-fos protein activation studies have also implicated the abnormal activity of OFC and prefrontal areas in compulsive drinking on SIP²³ (Merchan et al, unpublished). Second, the systemic administration of the D2/D3 receptors agonist quinpirole, which impairs reversal learning,⁵⁰ have also been shown to increase non-regulatory water intake in rats by contra-free loading, a proposed model of psychotic polydipsia.⁷⁰⁻⁷² Third, serotonin depletion has been shown to increase behavioral inflexibility.^{68,73} Furthermore, a low tryptophan diet has shown to increase compulsive drinking in HD rats on SIP (Merchan et al, unpublished). Finally, in a recent study, Barlow et al⁷⁴ found that highly perseverative rats in a spatial-discrimination serial reversal learning showed reductions in both serotonin 5-HT_{2A} receptor binding in the OFC and tryptophan hydroxylase

levels in the dorsal raphe nucleus, thereby suggesting that the serotonergic tone might be an endophenotype that predisposes animals to behavioral inflexibility and other forms of compulsive behavior. Similarly, HD compulsive rats on SIP have also shown reduced serotonin 5-HT_{2A} receptor binding and alterations in 5-HT tone in comparison to LD rats.³⁹

Myelination in HD Compulsive Rats

HD rats, selected by SIP, showed less myelination in the corpus callosum, striatum and basolateral amygdala in comparison with LD animals. In experimental studies, chronic treatment with the NMDA receptor antagonist MK-801 in mice have shown demyelination of the corpus callosum with schizophrenia-like behaviors, including hyperlocomotor activity and anxiety.^{75,76} This pharmacological model of schizophrenia has also been shown to increase compulsive drinking in rats on SIP.²¹ Moreover, animal models of schizophrenia, based on maternal immune activation, have also shown a reduction in myelinated fibers in the BLA amygdala, as well as long-lasting prepulse inhibition (PPI) deficits, locomotor hyperactivity and working memory deficits.⁷⁷ Reduced myelination in the corpus callosum and reduced oligodendrocyte density in the basolateral amygdala have been observed in schizophrenic patients.^{78–82} A possible explanation, for the less myelination observed in the HD rats, could be related with the neuroadaptation induced by the hyponatremic state observed in human PP. However, this might be discarded because: first, the findings of reduced myelination in schizophrenic patients cited above did not report PP; and second HD rats did not show symptoms associated with hyponatremia, such as disorientation, ataxia or seizures after SIP; moreover the regulatory levels of drinking in HD rats are not different from LD rats in the home-cage.⁸³ In contrast, the compulsive water ingestion by SIP in rats has demonstrated to induce neuroadaptation by the increase in the spine density in the dorsolateral striatum neurons,⁸⁴ pointing towards a neuronal modification on habit formation brain structures after SIP. Further research is needed to clarify the relation between polydipsia, schizophrenia, and reduced brain myelin, where polydipsia could be acting as a symptom or maybe as a vulnerability factor in psychotic episodes.

In conclusion, HD rats that were selected by SIP showed a reduced LI effect, behavioral inflexibility and reduced brain myelination, which are well known behavioral and biological markers of schizophrenia. Our data confirm previous findings that propose that SIP is an animal model of compulsive behaviors associated with schizophrenia and extends knowledge of the possible compulsive phenotype of HD rats that are selected by SIP. Future studies on the compulsive phenotype of HD rats that are selected by SIP can contribute to elucidating possible explanations for the comorbidity of different compulsive behaviors and

schizophrenia-like deficits in the same individual. This will improve our understanding of the mechanisms of vulnerability in compulsive spectrum disorders.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin* online.

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