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Downregulation of Oligodendrocyte Transcripts is Associated with Impaired Prefrontal Cortex Function in Rats

Justin R. Gregg, B.S.[1], **Nicole R. Herring, Ph.D.**[1], **Alipi V. Naydenov, B.S.**[1], **Ryan P. Hanlin, B.S.**[1], and **Christine Konradi, Ph.D.**[1,2,3,*]

[1]Departments of Pharmacology and Psychiatry, Vanderbilt University, Nashville, Tennessee, 37232

[2]Center for Molecular Neuroscience, Vanderbilt University, Nashville, Tennessee, 37232

[3]Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, Tennessee, 37203

Abstract

Abnormalities of brain white matter and oligodendroglia are among the most consistent findings in schizophrenia (Sz) research. Various gene expression microarray studies of postmortem Sz brains showed a downregulation of myelin transcripts, while imaging and microscopy studies demonstrated decreases in prefrontal cortical (PFC) white matter volume and oligodendroglia density. Currently, the extent to which reduced oligodendrocyte markers contribute to pathophysiological domains of Sz is unknown.

We exposed adolescent rats to cuprizone (CPZ), a copper chelator known to cause demyelination in mice, and examined expression of oligodendrocyte mRNA transcripts and PFC-mediated behavior. Rats on the CPZ diet showed decreased expression of mRNA transcripts encoding oligodendroglial proteins within the medial PFC, but not in the hippocampus or the striatum. These rats also displayed a specific deficit in the ability to shift between perceptual dimensions in the attentional set-shifting task, a PFC-mediated behavioral paradigm modeled after the Wisconsin Card Sorting Test (WCST). The inability to shift strategies corresponds to the deficits exhibited by Sz patients in the WCST. The results demonstrate that a reduction in oligodendrocyte markers is associated with impaired PFC-mediated behaviors. Thus, CPZ exposure of rats can serve as a model to examine the contribution of oligodendrocyte perturbation to cognitive deficits observed in Sz.

Keywords

oligodendrocytes; attentional set-shifting task; prefrontal cortex; hippocampus; schizophrenia; adolescence

Correspondence to: Christine Konradi, Ph.D., Vanderbilt University, Department of Pharmacology, MRB 3, Room 8160, 465 21st Avenue South, Nashville TN 37232-8548; USA, Tel. 615-936-1021, Fax 615-936-0484, E-mail: christine.konradi@vanderbilt.edu.

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Introduction

One of the most consistent observations in schizophrenia (Sz) research is an abnormality of white matter and oligodendroglia in the prefrontal cortex (PFC) and other brain areas (Davis et al., 2003; Karoutzou et al., 2008). Decreases in oligodendrocyte density were found in post-mortem studies (Uranova et al., 2001; Uranova et al., 2007; Vostrikov et al., 2007), a down-regulation of myelin genes was demonstrated in molecular studies (Hakak et al., 2001; Haroutunian et al., 2007; Mimmack et al., 2002), and brain imaging studies in living patients showed decreases in PFC white matter volume (Breier et al., 1992; Buchanan et al., 1998; Sanfilipo et al., 2000; Sigmundsson et al., 2001). White matter abnormalities have been observed at illness onset and in drug-free patients, suggesting that these changes are not due to medication effects (Bagary et al., 2003; Hakak et al., 2001).

Although symptoms associated with Sz are wide-ranging, recent emphasis has turned to cognitive deficits. This emphasis is driven by studies showing that cognitive function is one of the most critical determinants of quality of life for patients (Green et al., 2000). Many of the cognitive deficits are mapped to the PFC (Barch, 2005; Boeker et al., 2006), and Sz patients perform poorly on tasks that depend on PFC function, such as the Wisconsin Card Sorting Test (WCST), (Franke et al., 1992). To date, the extent to which reduced oligodendrocyte markers contribute to particular pathophysiological aspects of Sz and related disorders is unknown, although it is known that white matter is important for cognitive function (Dwork et al., 2007; Fields, 2008). Moreover, disorders associated with demyelination, such as metachromatic leukodystrophy or multiple sclerosis (MS) are frequently accompanied by psychosis (Feinstein, 2007; Hyde et al., 1992).

A number of rodent models were developed that induced PFC deficits through brain lesions, drug exposure and genetic preparations such as transgenic mice (for an in-depth review see Carpenter and Koenig, 2008). Many of these models cause severe pathologies beyond what is observed and expected in Sz. A specific model of the effect of oligodendroglial abnormalities on PFC function has not been examined to date.

Cuprizone (CPZ; biscyclohexanone oxalyldihydrazone) is a copper (Cu) chelator (Messori et al., 2007) which is used in mice to model demyelination disorders (Matsushima and Morell, 2001). Cu is an important catalytic and structural cofactor in a wide array of enzymatic processes with a narrow range of optimal concentration (Kim et al., 2008). Cu levels outside this range are highly neurotoxic (de Bie et al., 2007; Harrison et al., 2000; Menkes et al., 1962; Prohaska and Smith, 1982; Puig and Thiele, 2002). In mice, oral CPZ administration increases Cu and Zn concentrations in the brain (Zatta et al., 2005) and decreases the expression of myelin-specific genes (Morell et al., 1998; Pasquini et al., 2007; Seiwa et al., 2007). However, it is still not clear if CPZ-toxicity results from increased or decreased availability of Cu. Moreover, the exact reasons for the specific toxicity to myelin are still under investigation.

CPZ impairs spatial working memory in mice, which can be reversed by the antipsychotic drug quetiapine (Xiao et al., 2008). Although CPZ works well as a demyelinating agent in mice and as a model of MS, it is not used as a demyelinating agent in rats as it does not cause severe oligodendrocyte toxicity in this species (Adamo et al., 2006; Love, 1988; Matsushima and Morell, 2001; Purves et al., 1991).

The present experiments were designed to examine if CPZ exposure during adolescence reduces myelin transcripts in the rat comparable to what is observed in Sz, and the potential effect this has on PFC-mediated performance. We were interested in inducing a mild lesion of oligodendrocytes in the forebrain without inducing MS-like pathology.

Sprague-Dawley rats were exposed to a CPZ diet and gene expression patterns were examined in the PFC, hippocampus and striatum. The behavior of CPZ-treated rats was investigated in the Attentional Set Shifting Task (ASST), a modified version of the WCST which reveals impairments in Sz (Franke et al., 1992; Haut et al., 1996). One phase of the ASST, the extra-dimensional shift, is impaired by bilateral lesions of the medial frontal cortex and could thus reveal a decline in PFC function (Birrell and Brown, 2000; McAlonan and Brown, 2003).

The results demonstrate that CPZ exposure of the rat leads to a downregulation of myelin transcripts in the PFC and to decreased performance in the extra-dimensional shift of the ASST, while hippocampus and striatum are unaffected. This model might be useful to examine the contribution of oligodendrocytes to the cognitive deficits in various neuro-psychiatric disorders.

Materials and Methods

Subjects

Male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA), n=12 for gene expression microarrays and Q-PCR on P43, n=11 for Q-PCR on P54, n=11 for locomotor activity, and n=24 for ASST, were housed in groups of 2–4. Beginning on P29 and throughout all experiments, rats were fed CPZ (0.2%) diet or control diet (custom manufactured by Harlan Teklad, Madison, WI). Rats examined in the ASST were maintained on a restricted diet of 16–25 g of food per rat per day, starting three days before behavioral testing (P44) until the end of testing (P57), with water available *ad libitum* (Figure 1A). Animals were weighed tri-weekly, and chow was adjusted to maintain weight during the food restriction phase (Figure 1B). All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and Vanderbilt University's Institutional Animal Care and Use Committee guidelines.

Gene expression microarrays and Q-PCR

One group of animals was killed on P43 (day 15 of CPZ administration), and a second group on P54. The brains were extracted and snap-frozen at –30°C in 2-methylbutane, and stored at –80°C. Medial PFC [cingulate cortex 1, prelimbic cortex, and infralimbic cortex; from bregma anterioposterior (AP), +4.2 to +2.2] (Paxinos and Watson, 1986), hippocampus [from bregma AP −0.8 to −4.3] and striatum [caudate putamen; from bregma AP +1.2 to −0.8] (Paxinos and Watson, 1986) were dissected on a freezing microtome using micropunches. Care was taken to avoid inclusion of the corpus callosum in the punches.

Gene array experiments—Samples from individual rats (P43) were hybridized to individual arrays. RNA was extracted from 10–15 mg of tissue using the PureLink kit (Invitrogen, Carlsbad, CA). Two μg of total RNA was used for complementary DNA (cDNA) synthesis with the SuperScript double-stranded cDNA synthesis kit (Invitrogen Corp., Carlsbad, CA) and an oligo (dT) primer with T7 promoter sequence. In vitro transcription and biotinylation were carried out with the Gene Chip Expression 3′ Amplification kit for IVT (Affymetrix, Santa Clara, CA). Biotinylated RNA was hybridized to the RAE230 2.0 array (Affymetrix), and washing and staining were performed according to company protocol.

Quality control criteria—Tissue preparation and RNA extractions were performed in a single batch by the same investigator. All quality control criteria defined by Affymetrix were met by the samples, and no significant differences between experimental groups were observed. For the PFC, noise was 1.38 ± 0.1, % present call 63.4 ± 1.6, and 3'/5' GAPDH 1.21 ± 0.04 (average ± SD); for the hippocampus, noise was 1.16 ± 0.1, % present call 63.0 ± 2.1, and 3'/5' GAPDH 1.15 ± 0.04 (average ± SD). Quality control criteria were furthermore examined

in dChip (Li and Wong, 2001) and in RMA (Bolstad et al., 2003). Percent array outlier in dChip was 0.54 ± 0.1 for PFC, and 0.19±0.21 for hippocampus. One PFC sample with 3% array outliers, and one hippocampus sample with 24% array outliers were excluded from the analysis.

Q-PCR—cDNA was synthesized from 0.2 – 2 ug of total RNA from medial PFC, hippocampus and striatum (P43 and P54) using the iScript cDNA synthesis kit. A primer set for each gene was designed with the Primer3 software (www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi), for amplicons of 100–250 base pairs. Melt curve analysis and polyacrylamide gel electrophoresis were used to confirm the specificity of each primer pair. The iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) was used with an Opticon 2 real-time PCR detection system (Bio-Rad). Sample volume was 20 µl, with 4 µl of 1:10 diluted cDNA samples and 0.3 µM primers. The PCR cycling conditions were initially 95°C for 5 min followed by 39 cycles of 94°C for 10 s, 57°C for 15 s, and 72°C for 20 s. Data were collected between 72 and 84°C depending on amplicon melt temperature. A melt curve analysis was performed at the end of each Q-PCR experiment. Standard curves were generated for each primer pair in every experiment by diluting cDNA from a vehicle sample to a final concentration of 1.00, 0.2, 0.04, and 0.008. Blanks were assessed with each dilution curve to control for cross-contamination. Duplicates were used for dilution curves, blanks, and samples. Reported values were normalized to the average of three internal standards: beta-actin (Actb; GeneID: **81822**), alpha 1A tubulin (Tuba1a; GeneID: **64158**), and general transcription factor IIB (Gtf2b; GeneID: **81673**) none of which were regulated by gene-array or showed group differences in Q-PCR analysis. Genes of interest and primer pairs are listed in supplemental table S1.

Locomotor activity and Attentional Set-Shifting Task

For all behavioral experiments, investigators were blinded for treatment group. Three separate cohorts of animals were tested.

Locomotor Activity—Rats were tested for one hour on P43 and P57 (day 15 and 29 of exposure to CPZ; prior to and after ASST and food restriction). Activity was recorded in 43.2 × 43.2 × 50 cm chambers with photodetector-LED detection in sound-attenuated chambers (Med Associates, St. Albans, VT). All animals were assessed for locomotor activity between 1100–1400h. Chambers were cleaned with 70% ethanol between animals.

Attentional Set-Shifting Task (ASST)—The ASST was employed to assess PFC function as described previously, with modifications (Black et al., 2006). Rats were first trained to discriminate texture and then shifted to odor discrimination. The testing apparatus was a semitranslucent plastic bin (63.5 × 41.9 × 23.2 cm) that was divided into two equal-sized compartments (31.5 × 41.9 cm), separated by a nontransparent divider. One compartment contained two terracotta pots adjacent to each other. The bottoms of all pots were filled with paraffin approximately 12 mm high, and covered by a layer of Frosted Cheerios (General Mills, Minneapolis, MN) held in place by a mesh screen which made the Cheerios inaccessible to the rat. This was done to match potential ‘Cheerio odor’ in all pots. Each pot contained distinct digging medium, and the reinforced pot contained an accessible buried food reward (half, stale, Frosted Cheerio). During scent trials, the pot and media were rubbed with a perfume oil (The Body Shop, Wake Forest, NC). Pairs of media were composed of identical material differing in shape or size to ensure that the smell of the material was not used for discrimination. Each phase of the ASST had unique exemplars (Table 1). During the task, a rat was placed in the empty compartment and the divider was raised to allow access to both pots.

Beginning on P47 (day 19 of CPZ administration), after three days of food restriction, rats were habituated to chambers and trained to dig for five consecutive days (trial max/day = 30 min).

For dig training, a single unscented pot filled with polypropylene pellets was continuously re-baited until the rat approached and dug in less than 10 seconds after the divider was raised. A "dig" was defined as any distinct displacement of the digging medium with either the paw or the nose. Thus, a rat could investigate the pot or the medium in the pot by sniffing or touching the medium with its whiskers, before executing what was scored as a dig response.

On P54, in the *Introduction to Exemplars*, rats were trained to dig in Eppendorf tube lids for the food reward and to ignore Eppendorf tube bottoms which were not rewarded. On P55, rats performed a *Simple Discrimination* (SD; Table 1). The first four trials of a session were defined as exploratory trials, where the rat was permitted to dig in both pots, regardless of whether the first choice was correct. During subsequent trials, once a "dig" was executed in one pot, the other pot was removed to prevent digging in both pots within one trial. The criterion for each phase was six consecutive correct trials.

After reaching criterion in SD, rats were challenged with a *Compound Discrimination* (CD) task by introducing the second perceptual dimension, odor. The same medium as in SD was reinforced, with the intent to have the rats ignore the ‘odor’ dimension. After completion of the CD, a stimulus reversal (CD-Rev) was introduced, in which the previously non-enforced medium carried the reward (Table 1).

The *Intra-dimensional Shift* (IDS) started on P56. During this phase of the task, novel stimuli were presented, with medium still as the relevant perceptual dimension. After the IDS, rats performed a stimulus reversal (IDS-Rev), followed by an *Extra-dimensional Shift* (EDS). In the EDS, a novel set of stimuli was presented, but the previously irrelevant perceptual dimension, odor, became the relevant dimension. The final discrimination was an odor stimulus reversal (EDS-Rev). Of the 24 rats, trained in three different groups, six did not learn to dig (control n=2, CPZ n=4) and could not be used for the task. This difference was not significant for treatment group (Fisher’s Exact Test p=0.640).

Data analysis

For gene array experiments, a number of programs were used for data analysis: GeneChip Operating Software (GCOS) (Affymetrix) was used for scanning and to obtain quality control data. RMAExpress (Bolstad et al., 2003; Irizarry et al., 2003) was used for quantile normalization and background correction to compute expression levels for all probe sets. DNAChip Analyzer (Li and Wong, 2001) was used for clustering and David 2.0 (Dennis et al., 2003) was used to group regulated genes into functional annotations. In addition we used GenMAPP (Salomonis et al., 2007) to built 1473 brain-specific gene groups (MAPPs) by categorizing all curated probe sets from the rat RAE 230 2.0 array, and the MAPPfinder program to analyze regulation of groups. We used the permuted p-value (calculated with non-parametric statistic based on 2000 permutations of the data) as the indicator of significant regulation. Expression values were $\log_2$-transformed for statistical analyses.

T-tests and Wilcoxon Rank-Sum tests were performed for Q-PCR, for the individual phases of the ASST and for locomotor measures. Analysis of covariance was furthermore carried out for locomotor measurements. Repeated measures ANOVA was carried out for weight and food intake studies.

Results

Body Weights and Food Consumption

Between P29 and P44 no difference between treatment groups was observed in weight gain [$F_{(1,16)} = 1.0$, p=0.324]. During behavioral testing between P47 and P56, chow was adjusted for weight maintenance without weight gain [$F_{(1,16)} = 2.0$, p=0.179], (Figure 1).

Effects of CPZ exposure during adolescence on gene expression in the medial PFC, hippocampus and striatum

Gene expression microarrays—We chose gene expression microarrays for the initial molecular examination since they reveal broad, biological themes (Konradi, 2005). The RAE 230 2.0 Array contains over 31,000 probe sets. Of those, only probe sets with at least 40% 'Present' call in either group (control, CPZ), at least 1.2 fold difference in expression levels, and a p-value below 0.05 were used in the analysis. For the PFC, 337 probe sets matched these criteria, of which 149 were downregulated and 188 were upregulated. Using the NIH David Tool to examine the downregulated genes, one annotation cluster was above the minimum criteria. This annotation cluster had a high enrichment score (5.6) with the terms within that cluster related to myelination (Table 2; Figure 3B). Using our own 1473 brain-specific MAPPs and the MAPPfinder program (Salomonis et al., 2007), only 3 MAPPs reached significance, 'oligodendrocyte markers', 'myelin', and 'glia'. Table 3 lists the data for all regulated probesets of myelin markers, and these probesets clustered control and CPZ genes into distinct groups (Eisen et al., 1998), (Figure 2). False discovery rate of the group of myelin genes, established with 2000 permutations, was 0.0%.

Upregulated genes in the PFC fell into two annotation clusters, related to the somewhat generic Gene Ontology terms of 'stress' and 'development'. 'Development' encompasses terms like 'organ development', 'developmental process' and 'system development', and is among the most generic parent terms in the Gene Ontology database. 'Developmental process' contains over 20,000 gene products, 'organ development' almost 7,700. The genes in these categories did not seem to have a more specific function common to them. The genes in the second annotation cluster were related to stress response and included genes involved in inflammation (note that this group does not survive Benjamini-Hochberg correction) as well as the astrocyte marker glial fibrillary acidic protein (GFAP). In the MAPPfinder program, none of the brain-specific gene groups survived p-value permutation. Supplementary table S2 lists all regulated genes.

For the hippocampus, 194 probe sets matched the criteria, of which 115 were upregulated and 79 were downregulated. Neither set of genes was affiliated with any specific annotation in the DAVID database after Benjamini-Hochberg correction (Benjamini and Hochberg, 1995). Of the oligodendrocyte specific probe sets, only one, response gene to complement 32 (Rgc32), was downregulated (-23%; p=0.012); (Figure 3D). This finding was not significant for the myelin group (Fisher's exact test). GFAP was unchanged.

Nuclear transcripts coding for mitochondrial proteins were not changed in either brain area.

Q-PCR—Six genes were chosen for verification with Q-PCR (Figure 3). The five chosen oligodendrocyte markers were significantly downregulated in the medial PFC after 15 days of 0.2% CPZ treatment (MAG, t_{10}=-2.9, p<= 0.016; MBP, t_{10}=-4.1, p <= 0.002; MOBP, t_{10}=-4.2, p <= 0.002, MOG, t_{10} = −5.8, p <= 0.0002; PLP, t_{10} = −4.10, p <= 0.002) whereas the astroglial marker GFAP was significantly upregulated (t_{10} = 2.4, p <= 0.040), (Figure 3A; see Table 3 for abbreviations and Entrez Gene IDs). Comparable results were obtained with the gene arrays (Figure 3B; MAG, t_9= −5.6, p <= 0.0003; MBP, t_9= −6.4, p <= 0.0001; MOBP, t_9= −8.9, p <= 0.0001; MOG, t_9 = −5.8, p <= 0.003; PLP, t_9 = −8.1, p <= 0.0001, GFAP, t_9 = 3.3, p <= 0.009). A gene array analysis of the hippocampus (Figure 3D), or Q-PCR analyses of hippocampal or striatal mRNA, showed no changes (Figure 3C, Figure 3E). In the PFC, similar fold- reductions were observed after 26 days (P54) of exposure to 0.2% CPZ (Figure 3F; MAG, t_9= −3.2, p <= 0.01; MBP, t_9 = −2.2, p <= 0.05; MOBP, t_9 = −2.2, p <= 0.05; MOG, t_9 = −2.4, p <= 0.04; PLP, t_9 = −3.1, p <= 0.01, GFAP, p = ns). No changes were seen in these transcripts in a Q-PCR analysis of the hippocampus or striatum at P54 (data not shown).

Effects of adolescent CPZ exposure on locomotor behavior

Animals on the 0.2% CPZ diet and control animals were examined for locomotor activity in open field chambers on P43 (day 15 of CPZ exposure) and P57 (day 29 of CPZ exposure). No effect of treatment was observed between the groups on distance traveled or vertical movements measured in 5 minute intervals (ANOVA: distance traveled; vertical rearing; ANCOVA: treatment*time; Figure 4). An effect of 'time' was observed over the one hour period within each treatment group (P43: distance traveled $F_{(11,120)}$=79.9, p<=0.0001; vertical rearing $F_{(11,120)}$=54.6, p<=0.0001; P57: distance traveled $F_{(11,120)}$=222.3, p<=0.0001; vertical rearing $F_{(11,120)}$=187.6, p<=0.0001).

Effects of adolescent CPZ exposure on PFC-mediated behavior

Animals underwent behavioral testing using the ASST between P44 (16 days on CPZ) and P56 (28 days on CPZ). Only one phase of the task, the EDS, was significantly different ($t_{16} = -4.2$, p = 0.0007; z=3.0, p=0.003), whereas none of the other phases reached significance (Figure 5). Since bilateral lesions of the medial frontal cortex result in impairment in shifting of attentional sets the data indicate a *specific* impairment of the medial PFC (Birrell and Brown, 2000).

Discussion

The PFC in humans is important for a large range of functions, including working memory, action planning, response inhibition, decision-making, reward processing, and social behavior (Miller and Cohen, 2001; Ridderinkhof et al., 2004). Many of these functions are impaired in people suffering from Sz (Barch, 2005). Because a number of Sz studies have demonstrated decreased levels of oligodendrocyte mRNA transcripts and abnormalities of white matter in the PFC, we were interested if a reduction of myelin transcripts in the rat can cause behavioral deficits of the PFC similar to the ones observed in Sz. While such a study cannot address if the lower expression of oligodendrocyte markers in Sz is a neurodevelopmental or neurodegenerative event, it can establish a correlation between disturbances in myelin and specific Sz-related behavioral symptoms. Adolescence was chosen for treatment-onset with the Cu chelator CPZ, since Sz is frequently first diagnosed during this time (Mueser and McGurk, 2004).

CPZ affected oligodendrocyte transcripts in the PFC but not the striatum or the hippocampus with similar abnormalities at both the beginning, and toward the end of the ASST. The lack of effect of CPZ in the striatum and the hippocampus is surprising and can be explained by the low levels of CPZ used in the current study and the general resilience of rats to CPZ toxicity (Love, 1988; Matsushima and Morell, 2001; Purves et al., 1991). The data demonstrate that the PFC has a higher sensitivity to CPZ than the other two brain areas, which could be caused by a higher accumulation of CPZ in the PFC or a narrower tolerance for abnormal Cu levels.

The decrease in oligodendrocyte transcripts was accompanied by an increased difficulty to shift attention from one perceptual dimension to another in the EDS phase of the ASST. This is comparable to the deficit Sz patients show in the WCST (Berman et al., 1986; Franke et al., 1992; Haut et al., 1996). Attentional set-shifting has been linked to PFC function and is impaired by damage to the PFC in monkeys, rats and humans (Birrell and Brown, 2000; Dias et al., 1996a, b, 1997). The specificity for PFC involvement was demonstrated by the fact that the EDS was the only phase of the ASST that was affected by CPZ, since bilateral lesions of the medial frontal cortex should result in impairment in shifting of attentional sets, but not in impairments of initial acquisition or reversal learning (Birrell and Brown, 2000).

The observed upregulation of the astrocyte marker GFAP in the PFC is an indicator of astrocytosis. Astrocytosis was described in murine studies of the superior cerebellar peduncle after prolonged, high-dose CPZ administration (Ludwin, 1978). During demyelination, insulinlike growth factor-I (IGF-I) mRNA is induced in astrocytes while the IGF-I receptor is transiently expressed in oligodendrocytes, leading to the suggestion that astrocytosis might be beneficial for oligodendrocyte regeneration (Komoly et al., 1992). Indeed, astrocytes and the IGF-I system play an important role in the recovery from demyelination (Mason et al., 2003; Mason et al., 2000).

The upregulated group of genes in the PFC fell into generic Gene Ontology database categories related to ‘development’ and ‘stress’. The genes in these categories did not seem to have a specific function or location common to them, and more specific child terms of the generic parent terms such as ‘inflammatory response’ did not survive Benjamini-Hochberg correction. Thus, while it is quite plausible that stress-related genes are upregulated, these groups of genes would need further exploration.

Transcripts for mitochondrial genes were normal in the gene array analysis of PFC and the hippocampus, an important control since mitochondrial enlargement was described under high CPZ administration (Love, 1988), and CPZ is used to study megamitochondria in the mouse liver (Flatmark et al., 1980; Petronilli and Zoratti, 1990).

Given the history of CPZ as a model for MS in mice, we were particularly concerned about motor abnormalities. This concern was further fueled by a report that weanling Wistar rats exposed to CPZ at doses of 1% and 2% exhibited generalized weakness and wasting (Love, 1988). However, we did not observe motor impairments, either by direct observation, or by locomotor activity assessment. Likely explanations include the lower concentrations of CPZ used in our study and the different rat strain. Another concern in the present study involved sensory and sensory-motor deficits, since these modalities are important covariates in the ASST. Because CPZ rats performed comparable to control rats in six out of the seven phases of the ASST, and only showed a deficit in the medial PFC-mediated EDS, an impairment in tactile or odorant systems by CPZ is unlikely.

Despite the correlation between myelin abnormalities and behavior in the PFC, we cannot exclude that other brain areas are playing a contributory role in the behavioral effect. In mice, CPZ treatment affects the corpus callosum, the hippocampus, the cortex and the cerebellum (Blakemore, 1972, 1973; Hiremath et al., 1998; Remington et al., 2007; Skripuletz et al., 2008; Stidworthy et al., 2003), all of which can participate in memory processing (Vertes, 2006). While we did not see any effects on oligodendrocyte gene expression in the hippocampus and striatum, further clarification of the potential contribution of other brain areas to PFC function in the ASST might be useful in the future. Given that reduced levels of oligodendrocyte transcripts are observed in a number of different brain areas in Sz, this would not diminish the usefulness of the CPZ model (Haroutunian et al., 2007; Sokolov, 2007; Stewart and Davis, 2004).

Current treatments for Sz fall short on addressing impairments in cognitive function. One major challenge is to develop translational preclinical models that are subtle in their pathology and deal with cognitive deficits. Such models are needed to screen for drugs with potential benefits in the treatment of Sz. Our results provide such a translational model of PFC white matter deficits combined with cognitive-behavioral abnormalities, which could be useful for novel strategies of drug discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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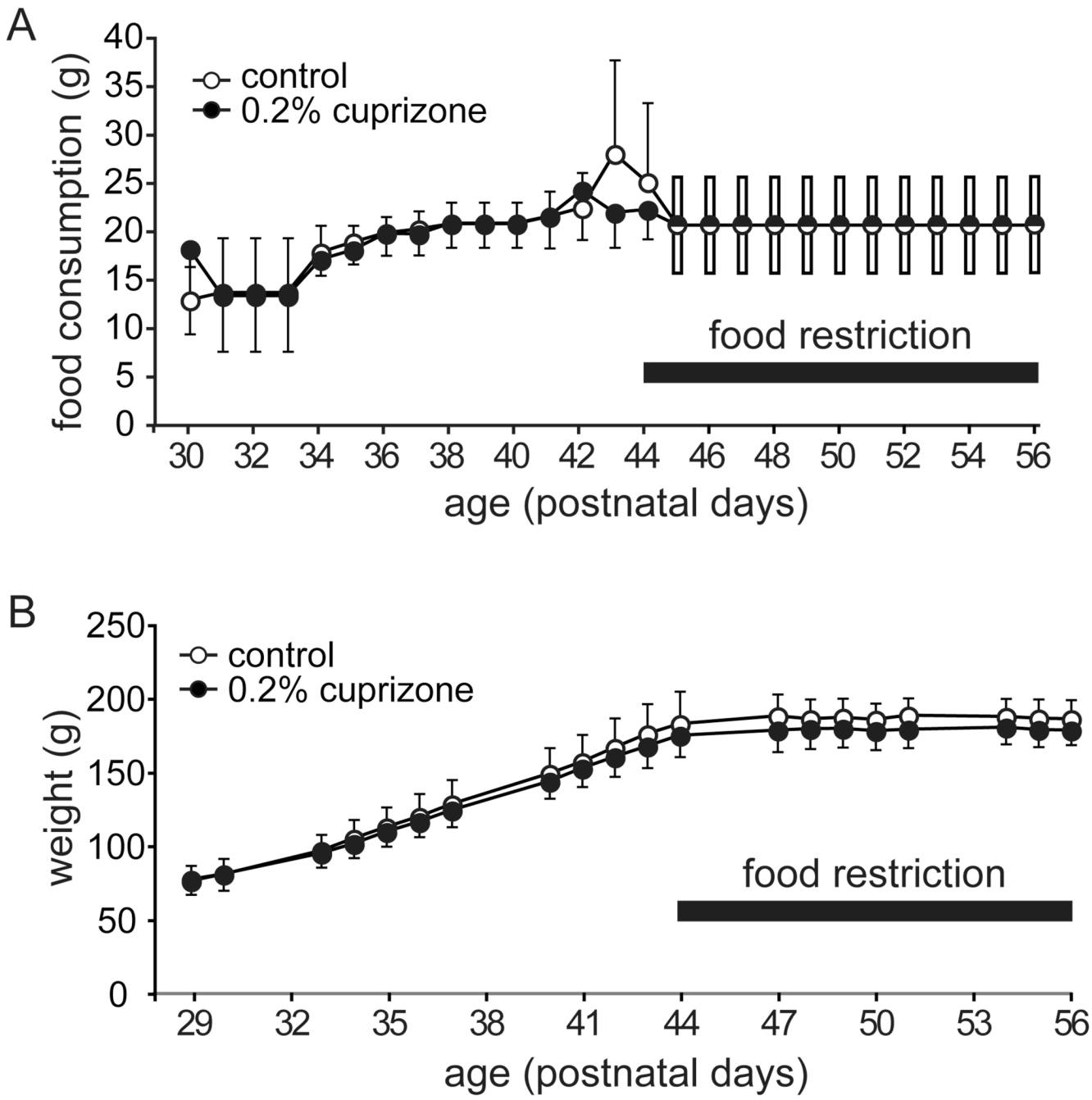

Figure 1.

CPZ intake and weight gain of ASST rats. A. Average food intake between P30 and P56. Chow was provided *ad libitum* until P44. Starting at P45, food restriction was implemented to increase motivation for food-digging in the ASST. During food restriction, enough food was provided to sustain weight. B. Representative growth curve for CPZ and control rats. Until P44, the growth curve was in line with the growth curve for male Sprague Dawley rats provided by Charles River Laboratories. All data in average ± SD of control (open circles; n=10) and CPZ-fed rats (black circles; n=8).

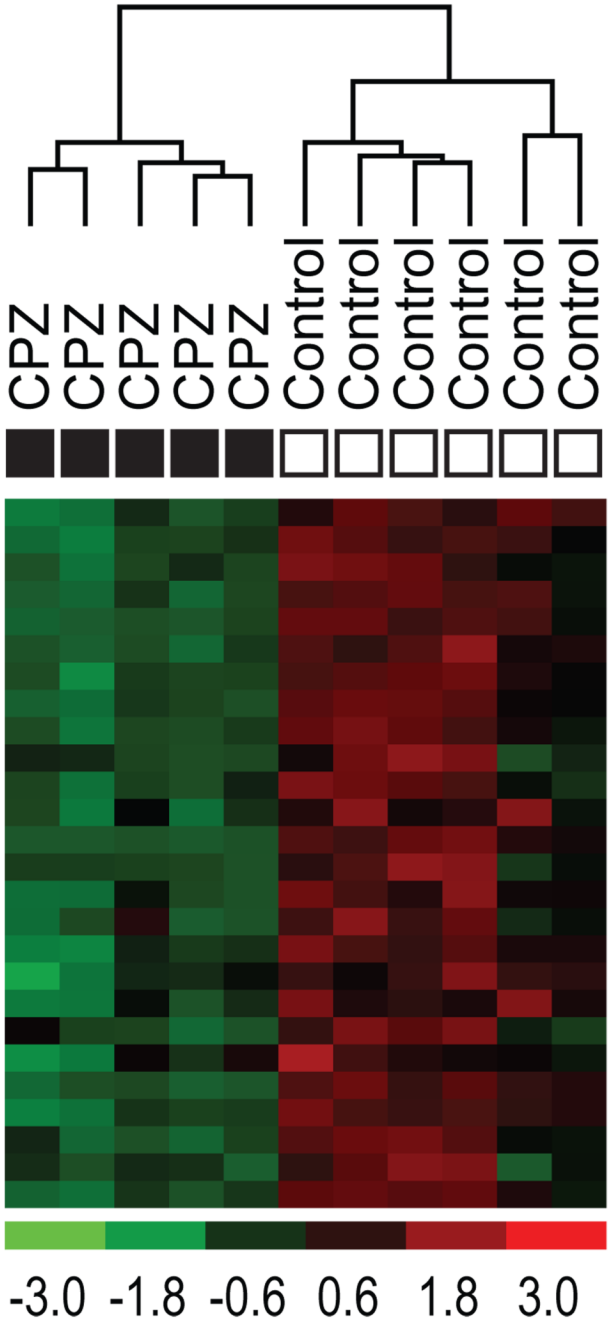

Figure 2.
Hierarchical clustering of PFC samples with probesets of myelin transcripts. Twenty-six probesets of myelin transcripts (Table 3) were used for hierarchical clustering of all samples used for gene array analysis. Samples clustered according to treatment group, demonstrating the specific effect of CPZ on the expression of oligodendrocyte markers.

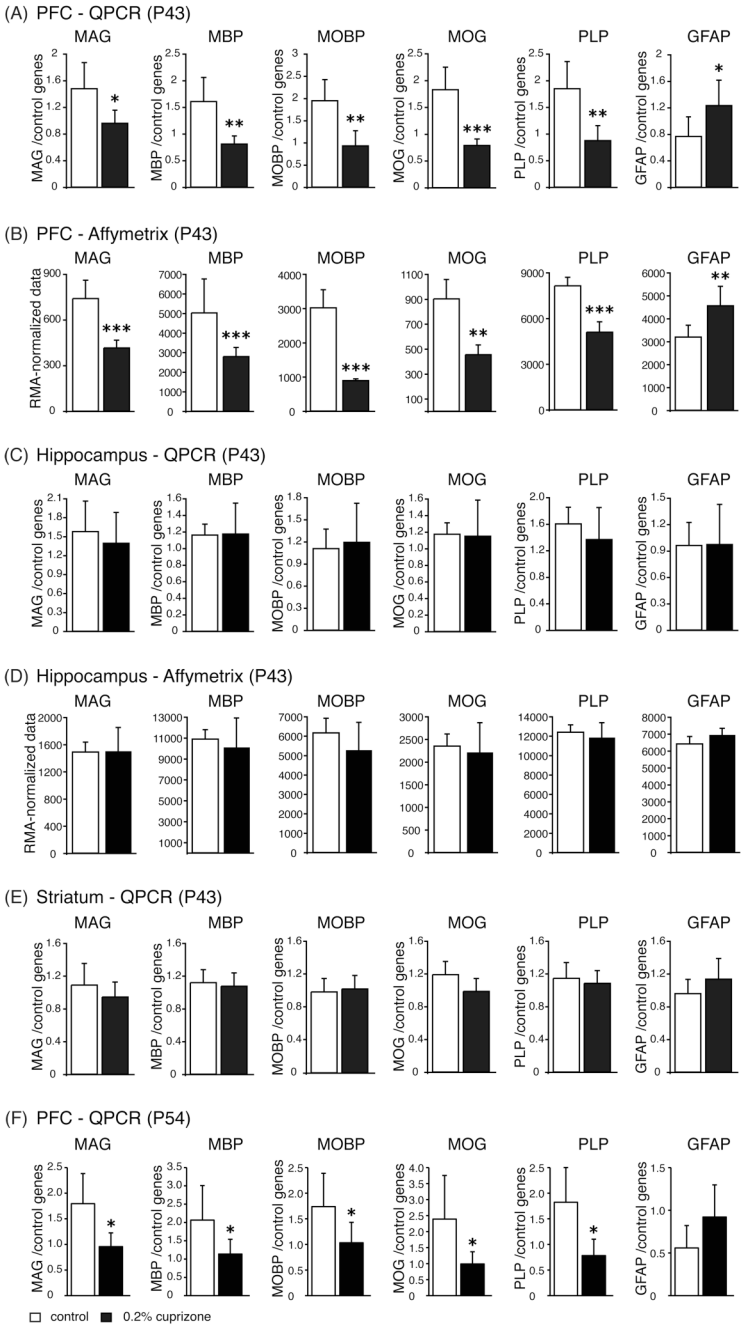

Figure 3.

Real time quantitative PCR results after 15 days of exposure to 0.2% CPZ in PFC (A), hippocampus (C) and striatum (E), and gene array results in PFC (B) and hippocampus (D) of control rats (open bars), and rats fed with CPZ chow (black bars). (F) Real time quantitative PCR results after 26 days of exposure to 0.2% CPZ in the PFC. For Q-PCR, all transcript levels were normalized to an average of 3 loading controls, β-actin (Actb), alpha-tubulin (Tuba1a) and general transcription factor IIB (Gtf2b). Mean normalized expression values of six independent samples per group + SD are plotted. *p<=0.05, **p<=0.01, ***p<=0.001. MAG, myelin-associated glycoprotein; MBP, myelin basic protein; MOBP, myelin-associated

oligodendrocytic basic protein; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein; GFAP, glial fibrillary acidic protein.

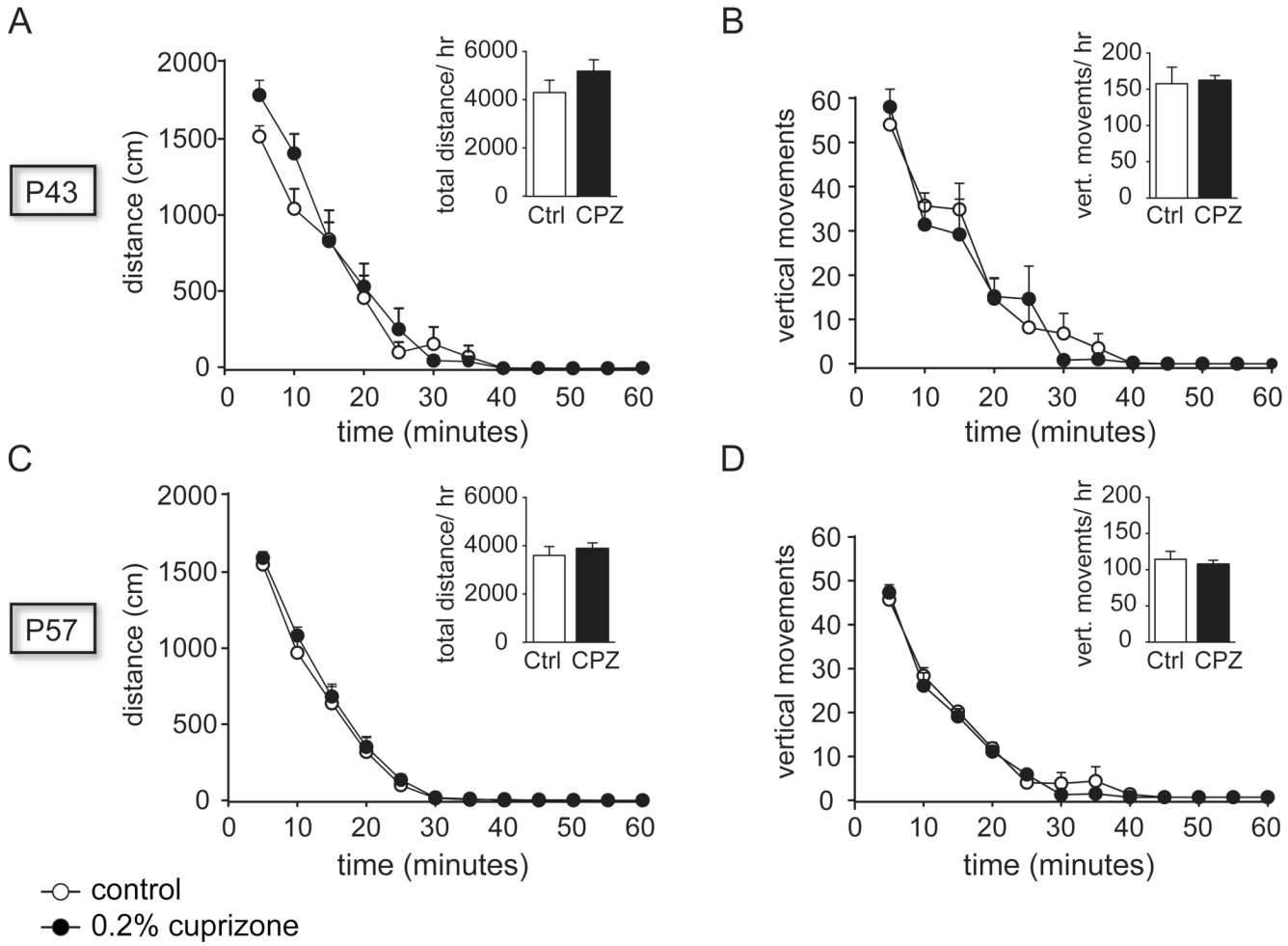

Figure 4.

Locomotor activity in the open field. Open field distance traveled was measured in 5 minute bins on P43, after 2 weeks of CPZ exposure, (A), and on P57, after 4 weeks of CPZ exposure and completion of the ASST, (C). Vertical movements were charted in 5 minute bins on P43, (B), and on P57, (D). All inserts show data accumulated over one hour. All data in average + SEM of control (open bars; n=6) and CPZ-fed rats (black bars; n=5).

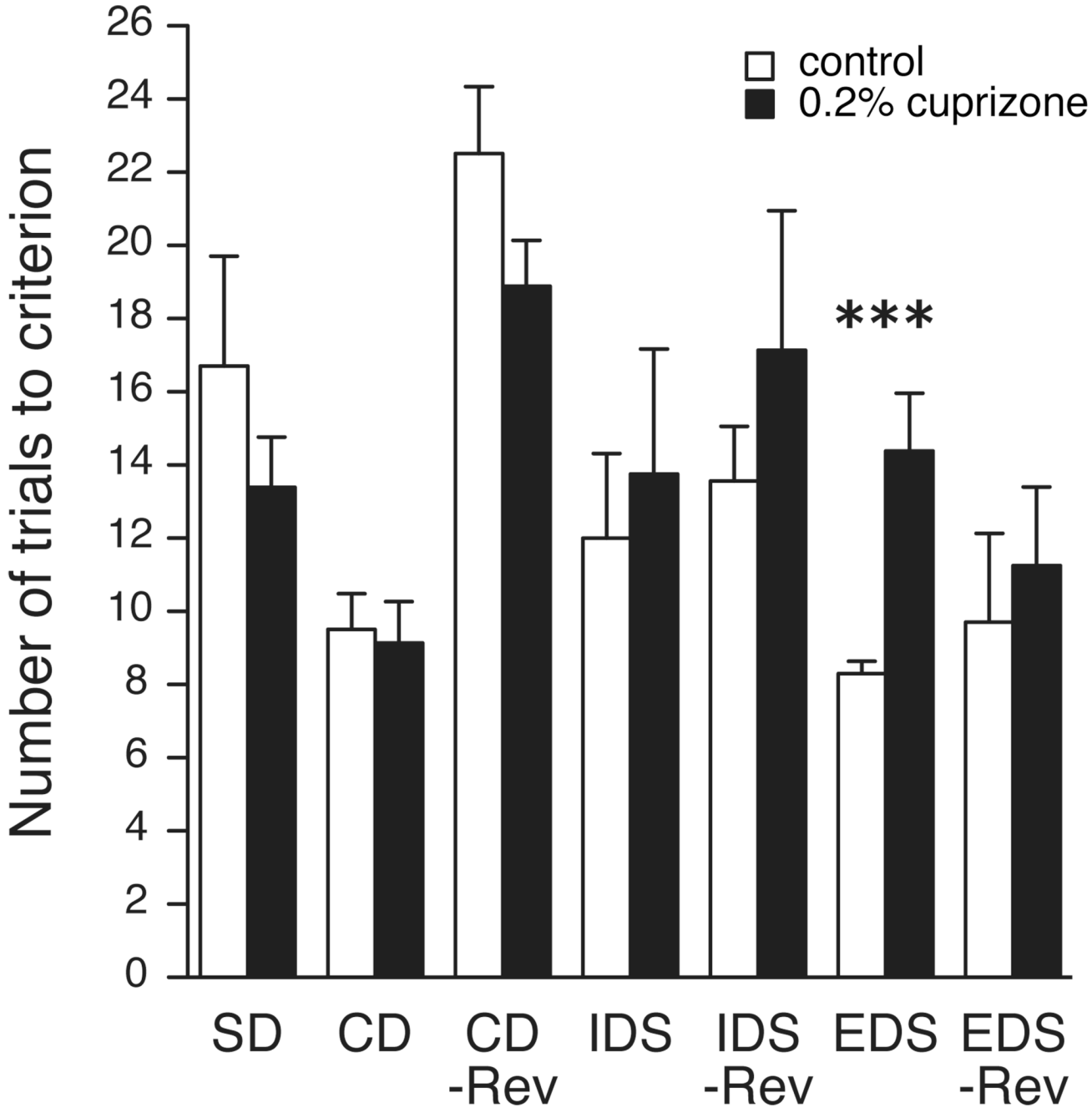

Figure 5.
Trials to reach criterion performance for all phases of the ASST. Values represent the mean + SEM of control (open bars, n=10) and CPZ-intake rats (black bars, n=8). ***p<=0.001. See Table 1 for abbreviations.

Table 1

Phases of the ASST, stimuli used, and age at which testing of rats was performed.

Phase (day)	Dimension		Combination of Stimuli		Age
	Relevant	Irrelevant	Positive	Negative	
Introduction to Exemplars	Medium	--	Eppendorf tube lids	Eppendorf tubes (w/o lids)	P54
Simple discrimination (SD)	Medium	--	Small chopped plastic tubing	Large chopped plastic tubing	P55
Compound Discrimination (CD)	Medium	Odor	Small chopped plastic tubing + Tea Rose Small chopped plastic tubing + White Musk	Large chopped plastic tubing + Tea Rose Large chopped plastic tubing + White Musk	P55
Compound Discrimination Reversal (CD-Rev)	Medium	Odor	Large chopped plastic tubing + Tea Rose Large chopped plastic tubing + White Musk	Small chopped plastic tubing + Tea Rose Small chopped plastic tubing + White Musk	P55
Intradimensional Shift (IDS)	Medium	Odor	Lg Glass Beads + Mandarin Lg Glass Beads + Patchouli	Sm Glass Beads + Mandarin Sm Glass Beads + Patchouli	P56
Intradimensional Shift Reversal (IDS-Rev)	Medium	Odor	Sm Glass Beads + Mandarin Sm Glass Beads + Patchouli	Lg Glass Beads + Mandarin Lg Glass Beads + Patchouli	P56
Extradimensional Shift (EDS)	Odor	Medium	Vanilla + Lg Foil Balls Vanilla + Sm Foil Balls	Jasmine + Lg Foil Balls Jasmine + Sm Foil Balls	P56
Extradimensional Shift Reversal (EDS-Rev)	Odor	Medium	Jasmine + Lg Foil Balls Jasmine + Sm Foil Balls	Vanilla + Lg Foil Balls Vanilla + Sm Foil Balls	P56

Table 2

Significant annotation clusters as calculated with NIH-David (39, 60). An enrichment score of '2' was used as cutoff. David provides batch annotation and gene term enrichment analysis to highlight the most relevant terms associated with a given gene list. The Functional Annotation Chart reduces the redundancy of annotations with related/identical terms by grouping similar annotations together. Although DAVID uses over 40 annotation categories, all significant enrichment scores came from the Gene Ontology (GO) database. The P-value in DAVID is a modified Fisher Exact P-Value. 'Benjamini' refers to the Benjamini-Hochberg procedure to control for the false discovery rate in multiple comparisons (61).

Annotation clusters for upregulated genes				
Annotation Cluster 1	**Enrichment Score: 5.1**	**Count**		
Database	**Term of the annotation cluster**	**# of genes involved in term**	**P_Value**	**Benjamini**
GO-Biological Process	organ development	31	6.60E-08	3.30E-04
GO-Biological Process	developmental process	47	4.70E-07	1.20E-03
GO-Biological Process	system development	35	5.00E-07	8.40E-04
GO-Biological Process	anatomical structure development	37	2.10E-06	2.10E-03
GO-Biological Process	cellular developmental process	31	8.70E-06	6.20E-03
GO-Biological Process	cell differentiation	31	8.70E-06	6.20E-03
GO-Biological Process	multicellular organismal development	36	1.10E-05	7.20E-03
GO-Biological Process	nervous system development	18	1.80E-04	7.30E-02
GO-Biological Process	multicellular organismal process	47	1.50E-02	7.90E-01
Annotation Cluster 2	**Enrichment Score: 3.5**	**Count**	**P_Value**	**Benjamini**
Database	**Term of the annotation cluster**	**# of genes involved in term**		
GO-Biological Process	response to stress	26	8.40E-07	1.10E-03
GO-Biological Process	response to external stimulus	19	1.70E-05	9.40E-03
GO-Biological Process	response to wounding	14	1.60E-04	7.10E-02
GO-Biological Process	inflammatory response	8	1.40E-02	7.70E-01
GO-Biological Process	response to stimulus	35	1.10E-01	9.90E-01
	Annotation clusters for downregulated genes			
Annotation Cluster 1	**Enrichment Score: 5.6**	**Count**		
Database	**Term of the annotation cluster**	**# of genes involved in term**	**P_Value**	**Benjamini**
GO-Biological Process	ensheathment of neurons	11	1.50E-13	3.80E-10
GO-Biological Process	axon ensheathment	11	1.50E-13	3.80E-10
GO-Biological Process	regulation of action potential	11	8.20E-13	1.40E-09
GO-Biological Process	myelination	10	3.90E-12	4.90E-09
GO-Biological Process	nervous system development	24	6.40E-11	6.40E-08
GO-Biological Process	system development	29	2.80E-07	2.40E-04
GO-Biological Process	transmission of nerve impulse	15	3.70E-07	2.70E-04
GO-Biological Process	anatomical structure development	31	5.50E-07	3.50E-04
GO-Biological Process	regulation of biological quality	19	2.40E-06	1.40E-03
GO-Biological Process	multicellular organismal development	29	1.10E-05	5.60E-03
GO-Biological Process	cell-cell signaling	16	1.20E-05	5.40E-03
GO-Biological Process	developmental process	34	2.60E-05	1.10E-02
GO-Biological Process	neurogenesis	9	2.10E-03	5.30E-01
GO-Biological Process	cellular developmental process	19	5.40E-03	8.00E-01
GO-Biological Process	cell differentiation	19	5.40E-03	8.00E-01
GO-Biological Process	cell development	13	4.90E-02	1.00E+00
GO-Biological Process	multicellular organismal process	33	6.70E-02	1.00E+00
GO-Biological Process	system process	20	7.40E-02	1.00E+00
GO-Biological Process	neurological system process	17	1.40E-01	1.00E+00
GO-Biological Process	cell communication	32	1.60E-01	1.00E+00

Table 3

Myelin markers found to be significantly regulated in CPZ treated rats. Fold-change was calculated with RMA. P-value was calculated with log2 transformed data. Affymetrix probe set IDs and Entrez gene ID numbers are provided. Transcripts shown in Figure 3 are denoted with 'x'.

Myelin markers (abbreviations)	fold change	P value (log2)	P call % of control	P call % of cuprizone	Entrez Gene ID	Affymetrix probe set ID	Verified with Q-PCR
myelin-associated glycoprotein (Mag)	−1.8	0.0002	100	100	29409	1368861_a_at	x
myelin-associated oligodendrocytic basic protein (Mobp)	−2.1	0.0000	100	100	25037	1368263_a_at	x
myelin-associated oligodendrocytic basic protein (Mobp)	−3.5	0.0025	100	100	25037	1370500_a_at	
myelin-associated oligodendrocytic basic protein (Mobp)	−3.4	0.0000	100	100	25037	1370434_a_at	
myelin basic protein (Mbp)	−1.8	0.0254	100	100	24547	1387341_a_at	x
myelin basic protein (Mbp)	−1.4	0.0002	100	100	24547	1368810_a_at	
myelin oligodendrocyte glycoprotein (Mog)	−2.0	0.0002	100	100	24558	1398257_at	x
myelin oligodendrocyte glycoprotein (Mog)	−1.3	0.0114	100	60	24558	1377352_at	
myelin and lymphocyte protein (Mal)	−3.8	0.0000	100	100	25263	1387040_at	
oligodendrocyte transcription factor 1 (Olig1)	−1.3	0.0005	100	100	60394	1387200_at	
plasma membrane proteolipid (Pllp)	−1.3	0.0138	100	100	64364	1386943_at	
proteolipid protein (Plp)	−1.6	0.0006	100	100	24943	1387112_at	x
claudin 11 (Cldn11)	−1.9	0.0305	100	100	84588	1369609_at	
claudin 11 (Cldn11)	−1.5	0.0029	100	100	84588	1376711_at	
CD9 antigen (Cd9)	−1.3	0.0119	100	100	24936	1371499_at	
v-erb-b2 erythroblastic leukemia viral oncogene homolog (Erbb3)	−1.6	0.0037	100	100	13867	1377821_at	
gelsolin (Gsn)	−1.4	0.0007	100	100	296654	1371414_at	
kallikrein 6 (Klk6)	−2.8	0.0000	100	40	29245	1368384_at	
response gene to complement 32 (Rgc32)	−1.3	0.0001	100	100	117183	1368080_at	
developmentally regulated protein TPO1	−1.2	0.0479	100	100	170907	1386979_at	
transferrin	−1.6	0.0027	100	100	24825	1370228_at	
tetraspanin 2 (Tspan2)	−1.5	0.0002	100	100	64521	1368104_at	
tetraspanin 2 (Tspan2)	−1.4	0.0008	100	100	64521	1368105_at	
UDP galactosyltransferase 8 (Ugt8)	−2.3	0.0001	100	100	50555	1368858_at	
aspartoacylase (Aspa)	−1.4	0.0000	100	100	79251	1368563_at	
Astrocyte Marker							
glial fibrillary acidic protein (Gfap)	1.4	0.0128	100	100	24387	1368353_at	x