Corresponding decrease in neuronal markers signals progressive parvalbumin neuron loss in MAM schizophrenia model

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Abstract
Alteration in normal hippocampal (HPC) function attributed to reduced parvalbumin (PV) expression has been consistently reported in schizophrenia patients and in animal models of schizophrenia. However, it is unclear whether there is an overall loss of interneurons as opposed to a reduction in activity-dependent PV content. Co-expression of PV and the constitutively expressed substance P (SP)-receptor protein has been utilized in other models to ascertain the degree of cell survival, as opposed to reduction in activity-dependent PV content, in the HPC. The present study measured the co-expression of PV and SP-receptors in the dentate and dorsal and ventral CA3 subregions of the HPC in the methylazoymethanol acetate (MAM) rat neurodevelopmental model of schizophrenia. In addition, these changes were compared at the post-natal day 27 (PND27) and post-natal day 240 (PND>240) time points. Brains from PND27 and PND>240 MAM (n=8) and saline (SAL, n=8) treated offspring were immunohistochemically processed for the co-expression of PV and SP-receptors. The dorsal dentate, dorsal CA3 and ventral CA3 subregions of PND27 and PND>240 MAM rats demonstrated significant reductions in PV but not SP-receptor expression, signifying a loss of PV-content. In contrast, in the ventral dentate the co-expression of PV and SP-receptors was significantly reduced only in PND>240 MAM animals, suggesting a reduction in cell number. While MAM-induced reduction of PV content occurs in CA3 of dorsal and ventral HPC, the most substantial loss of interneuron number is localized to the ventral dentate of PND>240 animals. The disparate loss of PV in HPC subregions likely impacts intra-HPC network activity in MAM rats.

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Introduction
The most consistently reported cellular alteration observed in the brains of schizophrenia patients is the reduction in the expression of the calcium-binding protein, parvalbumin (PV) (Lewis et al., 2001; Beasley et al., 2002; Kalus et al., 2002; Zhang et al., 2002c; Hashimoto et al., 2003; Sakai et al., 2008; Fung et al., 2010; Konradi et al., 2011; Curley et al., 2013) in GABAergic interneurons. Disruptions in inhibitory networks resulting from reductions in PV-expressing interneurons are proposed to contribute to the hyperactivity and loss of evoked gamma rhythms observed in the prefrontal cortex and hippocampus (HPC) (Lewis and Moghaddam, 2006; Lisman et al., 2008; Lodge et al., 2009; Konradi et al., 2011). The expression of PV is dependent on neural activity (Patz et al., 2004; Jiang and Swann, 2005; Sun, 2009). Recently, it has been shown that reductions of PV measured post-mortem in the HPC of schizophrenia patients may not involve an overall loss of cells as opposed to a decrease in protein content (Zhang et al., 2002c; Konradi et al., 2011).

As observed in patients, in the methylazoxymethanol acetate (MAM) developmental rodent model of schizophrenia there is a reduction in PV-expressing interneurons within the ventral HPC, which is proposed to lead to an aberrant increase in dopamine activity in the ventral tegmental area (Lodge et al., 2009). Aberrant increases in HPC activity have been implicated in the emergence of symptoms in schizophrenia (Schobel et al., 2009; Small et al., 2011). Although a decrease in PV expression in the ventral subiculum of the HPC of MAM rats has been reported previously, there is support for functional heterogeneity between the different HPC subregions, suggesting that the alterations resulting from MAM treatment may not be uniform. In particular, the dentate and CA3 regions of the HPC are important for the disambiguation of similar contexts, or pattern separation (Leutgeb et al., 2007; Tamminga et al., 2010; Schmidt et al., 2012). There is evidence that normal context discrimination is impaired in patients with schizophrenia, perhaps resulting from alterations in normal activity within the dentate and CA3 subregions. Consequently,
assessing PV expression in the dentate and CA3 subregions in the MAM model would confirm pathological changes specific to these areas and potential resultant behavioral abnormalities reported in schizophrenia patients.

The substance P (SP) receptor protein is expressed exclusively on nonprincipal, GABAergic interneurons in the hippocampus (Sloviter et al., 2001). The co-expression of SP receptors and PV has previously been reported for the dentate, CA1, and CA3 subregions of the dorsal HPC (Asacsy et al., 1997; Sloviter et al., 2001) and alterations in the co-expression has been used to quantify changes in HPC interneuron number in animal models of epilepsy (Sloviter, 1991; Sloviter et al., 2001, 2003), in that substance P receptors are continuously expressed even when activity-dependent PV content is lost. Verifying the extent of PV-expressing interneuron survival by measuring the co-expression of SP and PV in the HPC in the MAM model would be consistent with the pathological findings reported in schizophrenia patients. Recent studies have shown in normal rats that there is a progressive increase in PV expression in the ventral subiculum of the HPC as well as PFC when comparing post-natal days (PND) 25–40 and PND 60–85 (Caballero et al., 2013, 2014). Consequently, there are likely alterations in the normal developmental expression of PV in the MAM rat.

For the present experiment, offspring of dams treated with MAM or saline (SAL) were perfused on post-natal day 27 (PND27) or post-natal day 240 (PND>240). Immunofluorescent double-labeling was used to assess the expression of PV and SP receptors in the dentate/CA3 subregions of the dorsal and ventral HPC. By examining PV expression at these time points, it could be determined whether there is an initial decrease in PV-expression during periadolescence that persists to adulthood, or alternatively, a progressive decrease in PV expression that is not observed until adulthood.

Methods

Experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. All immunohistochemical experiments were conducted in PND27 (n=8) and PND>240 (n=8) offspring of pregnant dams treated with either saline or methylazoxymethanol acetate (details provided below). Animals were housed in a temperature (22 °C) and humidity (47%) controlled environment with a 12-h light/dark cycle (lights on at 7 a.m.) with ad libitum access to both food and water.

Methazoxymethanol treatment

MAM administration was performed as described previously (Moore et al., 2006; Lodge and Grace, 2007; Lodge et al., 2009; Gill et al., 2011). Briefly, timed pregnant female Sprague–Dawley rats (Hilltop) were obtained on gestational day (GD) 15 and individually housed in ventilated plastic breeding tubs. MAM (20 mg/kg, i.p.) was administered on GD 17. Control dams received injections of saline (1 ml/kg, i.p.). Male pups were weaned on day 21 and pair-housed with litter mates for either 27 d or 8 months, after which time they were used for immunohistochemical experiments.

Immunohistochemistry

PND27 and PND>240 animals were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.85% saline followed by 4% paraformaldehyde. Two time points were selected based on previous work in our laboratory examining the impact of early pharmacological interventions (PND 31–40), e.g. diazepam treatment, on subsequent dopamine system pathology in adult MAM animals (>PND60) (Du and Grace, 2013). We have also shown that this early time point is associated with altered stress responses, e.g. increased vocalizations and blunted HPA axis activation in response to stressful stimuli, in MAM animals (Zimmerman et al., 2013). In addition, PND27 has been shown to coincide with an early developmental time point (PND25–45) associated with decreased PV expression in the ventral subiculum of the HPC and PFC, increased risk taking behaviors, reduced behavioral inhibition, and perseverative errors during instrumental learning compared to adult animals (>60PND) (Sturman et al., 2010; Andrzejewski et al., 2011; Caballero et al., 2013; Simon et al., 2013). Brains were removed and subsequently cryoprotected in 25% sucrose for 24 to 48 h prior to being frozen and sliced on a freezing microtome (35 μm/slice). Free floating sections were then processed for their expression of parvalbumin and substance P via immunofluorescence using techniques described previously (Cano et al., 2001; Card et al., 2006). Sections were incubated in a combination of donkey serum, 0.1% Triton X-100, and mouse anti-PV antibody (1:1000 dilution, Sigma) with rabbit anti-substance P receptor antibody (1:100 dilution, Millipore) for 24 h at room temperature. After washing 3×10 min in phosphate buffered saline (PBS), the sections were then incubated in a mixture of Cy3 and Alexa conjugated donkey anti-rabbit/mouse antiserum (Jackson Immunoresearch Laboratories) for 2 h. Subsequently, sections were washed 3×5 min in PBS prior to being mounted on microscope slides. Prior to cover slipping, microscope slides were rinsed in a series of increasing concentrations of ethanol (EtOH) followed by xylene (2 min/wash).

Immunofluorescence quantification

Slides were viewed under two different filter conditions for Cy3 and Alexa Fluor 488 on an OlympusBX51 microscope with a Hamamatsu Orca-ER camera. For cell
counts, focus was set on PV-positive neurons and digital images were obtained using SimplePCI6 software (Hamamatsu Corporation, Japan). Sequential images (from both left and right hemispheres over region of interest obtained from three slices separated by 210 μm; six samples total per subject) were taken at 10× and 20× magnification through equivalent areas of the CA3 and dentate subregions of the ventral and dorsal HPC of SAL- and MAM-treated animals. Sections were selected from equivalent locations (same anterior–posterior position relative to bregma) in MAM and SAL rats. Only neurons within the hilus region of the dentate gyrus and pyramidal cell layer of CA3 were quantified (Fig. 1). Image-editing software (Adobe Photoshop) was used to combine the obtained images into plates prior to manual quantification of immunopositive neurons (immunopositive neurons=signal to background>3:1). Statistical comparisons were made on the average cell counts of the six samples from each region of interest.

**Statistical analysis**

Two-way analysis of variance (ANOVA) was used to compare differences between the MAM and SAL groups, PND>240 and PND27 groups, and the possible interaction between age group and MAM treatment. Tukey’s post-hoc comparisons were conducted for significant main effects. All statistics were calculated using SigmaPlot.

**Results**

**Ventral HPC subfield-specific loss of PV and SP-receptor expression in PND>240 MAM animals**

We reported previously that MAM rats demonstrate reduced PV expression in the mPFC and ventral subiculum of the HPC (Lodge et al., 2009). The aberrant activation of the dopamine system in MAM rats has been attributed to alterations in HPC output resulting from the loss of the normal functionality of PV-expressing interneurons (Lodge and Grace, 2007; Lodge et al., 2009). However, potential subregional changes, in either content or cell number, within the ventral HPC of MAM rats have not been described. Given that SP receptors are expressed in PV neurons in an activity-independent manner, the co-expression of PV and the SP-receptor has been utilized to determine whether the loss of PV represented a reduction in cell number or a loss of PV-content (Sloviter et al., 2003). Unlike the PFC where there is a low-level of co-expression of PV and SP-receptors (Matute et al., 1993), the HPC is especially amenable for the quantification of both proteins. Consequently, in the present study we assessed changes in PV and SP-receptor expression in HPC subregions known to exhibit a high-degree of co-expression of the two proteins, the dentate and both ventral and dorsal CA3 subfields (Leranth and Nitsch, 1994; Sloviter et al., 2001) of MAM (N=8) and SAL (N=8) rats (Fig. 2). By measuring the co-expression of the two proteins, we determined whether PV changes represented a loss of protein content within intact neurons or an overall reduction in cell number.

Individual 2-way ANOVAs were performed to assess differences in PV expression, SP-receptor expression and % of neurons co-expressing both proteins. Unless otherwise stated, Tukey’s post-hoc comparisons were used. In the hilus of the dentate gyrus of the ventral HPC, MAM rats exhibited significantly fewer PV-expressing neurons than SAL rats that was dependent on age (Fig. 3a; main effect of treatment, F₁,₁₂=6.91, p<0.05 and treatment×age interaction, F₀,₁₀=7.41, p<0.001; post-hoc, p<0.05). PND27 SAL rats demonstrated significantly more PV-expressing neurons than PND>240 MAM animals (individual Student’s t test, t₁₀=2.25, p<0.05). In contrast, there was no difference in the number of PV-expressing neurons between PND27 SAL rats and PND27 MAM rats (p=0.13). This would suggest that there was a progressive loss of PV in MAM rats from PND27 to PND>240. In comparison, the dentate of PND27 SAL rats contained more SP receptor-expressing neurons than both PND27 and PND>240 MAM animals (Fig. 3b; main effect of treatment, F₁,₁₂=13.09, p<0.01; post-hoc, p<0.05). However, when considering the percent of cells...
co-expressing both PV and SP-receptors, only PND>240 MAM rats exhibited significantly fewer co-expressing neurons than PND27 SAL rats (Fig. 3c; main effect of treatment, $F(1,12)=6.33$, $p<0.05$; individual Student’s $t_{(6)}=2.63$, $p<0.05$). No significant differences were found in either SP receptor expression or percent of cells co-expressing the two proteins in regards to age or age × treatment interaction (main effect of age, $F(1,12)=2.04$, age × treatment interaction, $F(1,15)=0.01$ and main effect of age, $F(1,12)=0.74$, age × treatment interaction, $F(1,15)=0.02$, respectively; all $p’s>0.05$). Overall, these data suggest that the loss of PV in PND>240 MAM animals represents a loss of cell numbers as opposed to loss of PV content.

Similar to the dentate, MAM rats demonstrated significantly fewer PV-expressing neurons than SAL rats in the ventral CA3 subregion (Fig. 3d; 2-way main effect of treatment, $F(1,12)=5.67$, $p<0.01$; post-hoc, $p<0.05$). There were also no significant differences in PV expression in ventral CA3 with respect to age (main effect of age, $F(1,12)=0.10$, $p>0.05$). However, unlike the pattern observed in the dentate, there was no interaction between PV expression at the PND27 and PND>240 time points and MAM treatment ($F(1,15)=0.04$, $p=0.85$). Subsequent post-hoc comparisons failed to reveal any significant differences amongst the individual age groups, but there was a significant difference between the combined MAM and SAL groups (post-hoc, $p=0.03$). This would suggest a general reduction of PV expression in MAM animals independent of age. In contrast to the reductions in SP-receptor expression observed in the ventral dentate, there were no changes in SP-receptor expression in ventral CA3 in the MAM animals (Fig. 3e; main effect of treatment, $F(1,12)=3.97$, $p=0.07$), nor were there any differences in SP-receptor expression in ventral CA3 between the PND27 and PND>240 time points (main effect of age, $F(1,12)=0.46$, $p=0.51$). As expected from the pattern of reduced PV-expression, but unaltered SP-receptor expression in ventral CA3, the percent of cells co-expressing the two proteins did not significantly vary between MAM and SAL animals (Fig. 3f; main effect of treatment, $F(1,12)=0.94$, $p=0.35$). In addition, no significant differences were found in either SP receptor expression or percent of cells co-expressing the two proteins in regards to age or age × treatment interaction (main effect of age, $F(1,12)=0.004$, age × treatment interaction, $F(1,15)=0.46$ and main effect of age, $F(1,12)=0.007$, age × treatment interaction, $F(1,15)=0.19$, respectively; all $p’s>0.05$). Consequently, the loss of PV in ventral CA3 may represent a reduction in PV content and not necessarily a loss of PV neurons in MAM rats.

Fig. 2. Representative images of parvalbumin (PV) and substance P (SP)-receptor expression in the dentate and CA3 subregions of the ventral (a) and dorsal (b) hippocampus (HPC) of post-natal day (PND) 27 and adult saline (SAL) and methylazoxymethanol acetate (MAM)-treated offspring. Yellow arrows indicated neurons that were double-labeled for SP-receptors and PV.
Dorsal HPC reduction in PV but not SP-receptor expression in MAM animals

There are distinct behavioral functions attributed to the dorsal and ventral subregions of the HPC (Anagnostaras et al., 2001; Peleg-Raibstein and Feldon, 2006; Fanselow and Dong, 2010; Kesner et al., 2011). In addition, increased activation of the ventral, but not dorsal, HPC has been associated with increased activation of the dopamine system (Peleg-Raibstein and Feldon, 2006). We sought to determine whether reductions of PV-expression reported for the ventral HPC of MAM rats (Lodge et al., 2009) extended to the dorsal HPC as well.

In the hilus of the dentate gyrus of the dorsal HPC, MAM rats exhibited significantly fewer PV-expressing neurons than SAL rats (Fig. 4a; main effect of treatment, $F_{(1,12)}=23.09$, $p<0.01$; post-hoc, $p<0.05$). In addition, there were age-dependent differences in PV expression between SAL and MAM rats, but there was not a significant age×treatment interaction (main effect of age, $F_{(1,12)}=7.95$, $p<0.05$ and age×treatment interaction, $F_{(1,15)}=0.79$, $p>0.05$). At the PND27 time point, MAM rats already showed reduced PV-expression compared to PND27 SAL rats ($p<0.05$). PND>240 SAL rats exhibited fewer PV-expressing neurons than PND27 SAL rats ($p<0.05$). However, PND>240 MAM rats exhibited significantly reduced PV expression in the dorsal dentate than PND >240 SAL rats ($p<0.05$). This would suggest that although there was already an age-dependent reduction in PV expression in the dorsal dentate of normal rats, MAM rats demonstrated an even greater decrease. In contrast, there was no difference between SAL and MAM rats in the number of SP-receptor-expressing neurons in the dorsal dentate (Fig. 4b; main effect of treatment, $F_{(1,12)}=2.99$, $p=0.11$). In addition, when considering the percent of cells co-expressing both PV and SP-receptors, there was no difference between SAL and MAM rats (Fig. 4c; main effect of treatment, $F_{(1,12)}=0.67$, $p=0.43$). No significant
differences were found in either SP receptor expression or percent of cells co-expressing the two proteins in regards to age or age x treatment interaction (main effect of age, $F_{(1,12)}=0.28$, age x treatment interaction, $F_{(1,15)}=0.01$ and main effect of age, $F_{(1,12)}=1.47$, age x treatment interaction, $F_{(1,15)}=2.90$, respectively; all p > 0.05). Similar to what was observed in the ventral CA3 subregion, these data support a reduction of PV-content in neurons in the dorsal dentate of PND27 and PND>240 MAM rats, as well as PND>240 SAL rats. This is in contrast to the overall reduction in cell number observed in the ventral dentate of MAM rats. These data suggest that the decrease in PV in dorsal dentate in MAM rats may be due to decreased PV neuron drive, whereas the decrease in the ventral dentate is due to PV neuron loss, suggesting a greater vulnerability of neurons in the ventral dentate to MAM treatment.

Similar to the dorsal dentate, MAM rats demonstrated significantly fewer PV-expressing neurons than SAL rats in the dorsal CA3 subregion that was not dependent on age (Fig. 4d; main effect of treatment, $F_{(1,12)}=17.43$, p < 0.001; post-hoc, p < 0.05; main effect of age, $F_{(1,12)}=1.09$, p = 0.32). Both PND27 and PND>240 MAM rats displayed significantly fewer PV-expressing neurons in dorsal CA3 compared to SAL rats (post-hoc comparisons, p < 0.05, respectively). In addition, there were no changes in SP-receptor expression in dorsal CA3 in the MAM animals (Fig. 4e; main effect of treatment, $F_{(1,12)}=0.92$, p = 0.36), nor were there any differences in SP-receptor expression in dorsal CA3 between the PND27 and PND >240 time points (main effect of age, $F_{(1,12)}=0.55$, p = 0.47). As expected from the pattern of reduced PV-expression but unaltered SP-receptor expression in dorsal CA3, the percent of cells co-expressing the two proteins did not significantly vary between MAM and SAL animals (Fig. 4f; main effect of treatment, $F_{(1,12)}=0.65$, p = 0.44). No significant differences were found in PV expression, SP receptor expression, or percent of cells co-expressing the two proteins in regards to a potential age x treatment interaction (age x treatment interaction, $F_{(1,15)}=0.17$ and
age × treatment interaction, $F_{(1,15)} = 2.07$, respectively; all $p’s > 0.05$). Consequently, the loss of PV in dorsal CA3 likely represented a reduction in PV content in neurons in MAM rats.

**Discussion**

This is the first report of HPC subregional differences in PV and SP-receptor expression in the MAM model of schizophrenia. Previously, we demonstrated a reduction in PV expression in the ventral subiculum of the HPC (Lodge et al., 2009). We extend these findings of diminished PV expression to the dentate gyrus and both dorsal and ventral CA3 subregions of the HPC. Even though there is not complete overlap in those neurons expressing both SP and PV, there is a substantial proportion of neurons co-expressing the two proteins. As a consequence, reductions or increases in the co-expression would be indicative of a loss of cell number, as opposed to mere changes in an individual protein without alterations in their co-expression. As such, by evaluating the co-expression of SP-receptors and PV, we show that the loss of PV in the CA3, but not dentate, is likely a reduction of PV-content in surviving neurons. These data are consistent with studies from schizophrenia patients demonstrating HPC reduction in the relative density of PV-expressing neurons without any change in overall neuron number (Zhang et al., 2002c; Konradi et al., 2011). In contrast, the ventral dentate exhibited a significant decrease in neurons co-expressing SP receptors and PV, indicative of a loss of cell number. This would suggest that the impact of MAM on PV-expressing interneurons is not uniform across all HPC subregions and could have a broad impact on intra-HPC network activity. Importantly, the reduction in PV and SP-receptors was most prominent in the PND > 240 MAM animals. A progressive loss of these proteins into adulthood could signify a potential time point during perinatal changes that could potentially prevent the transition to schizophrenia in the adult (Du and Grace, 2013).

**Implications for pathological changes in ventral HPC and cognitive disturbances in schizophrenia**

The dorsal and ventral subdivisions of the HPC in the rat are homologous to the posterior and anterior portions, respectively, of the HPC in humans (Strange and Dolan, 1999; Sasaki et al., 2004). While some studies have reported simultaneous alterations in both anterior and posterior HPC areas of schizophrenia patients (Herold et al., 2013; Zierhut et al., 2013), there have also been reports of exclusive volumetric and activity-related changes in the anterior, but not posterior, HPC (Szczesko et al., 2003; Narr et al., 2004; Velakoulis et al., 2006; Schöbel et al., 2009). Evidence from rodents also suggests that PV expression in the ventral, but not dorsal, subiculum undergoes protracted changes during development (Caballero et al., 2013).

Numerous morphological and neurochemical alterations specific to the dentate gyrus region of the HPC have been observed in the post-mortem brain from schizophrenia patients (reviewed in Kobayashi, 2009). It has been suggested that the dentate gyrus may be especially vulnerable to developmental alterations due to continuous postnatal neurogenesis. Mossy fibers from the dentate gyrus convey processed entorhinal cortical input representing current contextual stimuli to CA3 (Treves and Rolls, 1992; O’Reilly and McClelland, 1994). Consequently, developmental abnormalities observed in the dentate can have downstream effects on the normal plasticity at the mossy fiber-CA3 synapses. Altered connectivity between the dentate gyrus and CA3 has been proposed to contribute to errors of pattern completion in schizophrenia patients and an inability to disambiguate between present and past experiences in memory (Tamminga et al., 2010). In addition, electrophysiological recordings from and assessment of immediate early gene activation in the dentate gyrus have substantiated a role for this HPC subregion in disambiguating between similar behavioral contexts (reviewed in Schmidt et al., 2012). Close examination of the mossy fiber-CA3 projection in schizophrenia patients reveals a reduction in spine number as well as spines forming synapses (Kolomeets et al., 2005). These data indicate a decreased efficacy of projections from the dentate to CA3 and an association with the presence of positive symptoms. Data from the current study confirm a significant loss of interneurons in the ventral dentate of PND > 240 MAM rats. Consequently, alterations in the normal connectivity between the dentate and CA3 resulting from this cell loss in MAM rats could lead to inadequate conveyance of current contextual stimuli to CA3, and may play a role in sensory processing or cognitive disturbances characteristic of schizophrenia.

There is evidence that alteration in the normal functionality of ventral, but not dorsal, HPC is more involved in some of the behavioral disturbances associated with schizophrenia. Prepulse inhibition (PPI) of the startle reflex, a measure of sensorimotor gating, is commonly disrupted in schizophrenia. Both electrical and chemical activation via N-methyl-D-aspartate (NMDA) of the ventral, but not dorsal, HPC can disrupt PPI in rodents (Klärner et al., 1998; Bast et al., 2001; Swerdlow et al., 2001; Zhang et al., 2002a, b; Howland et al., 2004). Schizophrenia patients also exhibit impaired performance during attentional set-shifting tasks (Pantelis et al., 1999). Temporary inactivation of the ventral, but not dorsal, HPC in rodents early in development can lead to impaired attentional set-shifting performance in adulthood (Brooks et al., 2012). Furthermore, there are indications that regionally specific alterations in PV in the HPC could underlie both the behavioral and oscillatory disturbances observed in schizophrenia. Genetic disruption of
normal antioxidant activity, a known susceptibility factor for schizophrenia, by interfering with glutathione synthesis results in selective decreases in PV expression in the dentate and CA3 subregions of the ventral, but not dorsal, HPC (Steullet et al., 2010). The reduction in PV coincides with altered beta and gamma oscillatory activity as well as impaired object recognition and delayed fear conditioning (Lodge et al., 2009; Steullet et al., 2010).

Consequently, the differential contribution of the ventral and dorsal HPC in generating the pathological behaviors observed in schizophrenia is compatible with the disparity in reduced co-expression of SP-receptors and PV observed in the present study.

It is important to note that there is a diverse array of interneuron subtypes, expressing either PV, somatostatin, cholecystokinin, neuropeptide Y, or vasopressin in both the dorsal and ventral HPC. While there are reports of reductions in other interneuronal markers, including somatostatin (Morris et al., 2008; Fung et al., 2010; Konradi et al., 2011) and cholecystokinin (Curley and Lewis, 2012), in schizophrenia patients, corresponding experiments in the MAM model have not been conducted. In addition, PV-expressing interneurons represent the largest population of interneurons in the HPC along with previously reported high co-localization with the SP-receptor (Sloviter et al., 2001). Reductions in PV have previously been reported in the MAM model in both the mPFC and ventral subiculum (Lodge et al., 2009).

Importantly, these reductions were associated with alterations in task-induced gamma oscillations during the latent inhibition paradigm, similar to what has been reported in schizophrenia patients (Spencer et al., 2003; Symond et al., 2005; Bucci et al., 2007; Williams et al., 2009; Koenig et al., 2012). Consequently, PV is the most suitable target when assessing immunohistochemical changes in the MAM model.

**Therapeutic implications for age-related changes in PV-expression in SZ**

The loss of PV- and SP-receptor expressing neurons in the dentate gyrus of MAM rats was most pronounced in the PND>240 animals. There is evidence from rodents and nonhuman primates that PV expression in the PFC as well as the ventral subiculum of the HPC increases across development (Fung et al., 2010; Caballero et al., 2013, 2014; Fish et al., 2013). It should be noted that in the present study the reduction of PV in the dorsal dentate in normal rats PND>240 might indicate that this region is more susceptible as a consequence of normal aging. PND>240 is at the early stage of observed decreases in neurogenesis in the dentate gyrus in adult rats, with the largest decreases occurring >360 (Kuhn et al., 1996; Rao et al., 2006). However, in the present study additional decreases in PV in the dentate were observed in the MAM animals at the same time point as SAL rats. Typically, post-mortem assessment of regional changes of PV expression in schizophrenia involves the comparison with age-matched control subjects. Currently, there is no definitive study examining the temporal pattern of PV expression changes in schizophrenia patients. However, a compelling recent study reported that reductions in PFC PV expression were not associated with the age or duration of illness of schizophrenia patients (Hoffman et al., 2013). When considered with the normal expected developmental increase in PV, this would suggest that there is a dampening of PV expression in schizophrenia. The data from the present study are consistent with the therapeutic potential of interventions introduced during adolescence in schizophrenia. Indeed, our lab has shown that treatment with diazepam during adolescence in MAM rats can prevent the abnormal increase in dopamine system activation observed in PND>240 animals (Du and Grace, 2013). Alternative animal models of schizophrenia have reported a similar benefit of antipsychotic drug treatment during adolescence in reversing various morphological and behavioral correlates of schizophrenia in adult animals (Piontkewitz et al., 2011, 2012). Of note, risperidone treatment can prevent the reduction in HPC PV-expression observed in the polyriboinosinic–polyriboycytidic acid model of schizophrenia (Piontkewitz et al., 2012). Perhaps by preventing the cell loss observed in the dentate gyrus of MAM rats, potential therapies could preserve the normal connectivity with CA3 and enable the subject to retain normal pattern separation behavioral functions.

**Conclusions**

While MAM-induced reduction of PV content occurs in the CA3 subregion of dorsal and ventral HPC, the most devastating loss of interneuron number is localized to the ventral dentate of PND>240 animals. These data suggest that PV loss in dorsal HPC may be attributable to decreased PV neuron drive, whereas in the ventral HPC it is due to PV neuron loss. Given that the pathology is more prevalent in the PND>240, these data suggest that early intervention may prevent the transition to psychosis later in life (Thompson et al., 2004; Du and Grace, 2013).

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**Statement of Interest**

Competing financial interests. Johnson and Johnson, Lundbeck, Pfizer, GSK, Puretech Ventures, Merck, Takeda, Dainippon Sumitomo, Otsuka, Lilly, Roche,
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