RESEARCH ARTICLE

Histamine and acetylcholine receptor involvement in sensorimotor gating: an autoradiography study [version 1; referees: 2 approved with reservations]

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Abstract

Sensory gating is a way by which the brain manages sensory information flow. For optimal allocation of neural resources, it is important to be able to screen out (or "gate") irrelevant sensory information when another stimulus is being processed. Sensorimotor gating more generally refers to the overall process of modulation of the motor responses to sensory stimuli. Impaired sensorimotor gating is seen in a variety of neurobehavioral disorders including schizophrenia, autism and sensory processing disorder. The degree of sensorimotor gating can be studied behaviorally by indexing prepulse inhibition (PPI). PPI reflects the degree of suppression of a startle response to an intense sensory stimulus when it is preceded by a more modest sensory stimulus. The neural circuitry underlying PPI has been shown to include dopaminergic and cholinergic systems. We previously found that histaminergic H1 receptors also play important roles in sensorimotor gating: the acute administration of the histamine H1 antagonist, pyrilamine, significantly reverses the PPI impairment caused by the NMDA glutamate antagonist, dizocilpine (MK-801). The current study was conducted to determine the anatomic bases for histaminergic and cholinergic regulation of the effect of NMDA antagonism on PPI. Using autoradiography, we found that pyrilamine treatment decreased H1 receptor binding in the anterior cingulate, which correlated with PPI improvement. Furthermore, we found that pyrilamine treatment resulted in increased α7-nicotinic acetylcholine receptor binding in the insular cortex, which also correlated with PPI improvement. These findings shed light on the interaction between histamine and acetylcholine signaling in a distributed network of PPI modulation.

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Introduction

Sudden intense sensory stimuli can evoke a rapid muscular reaction called the startle response. The startle response can be inhibited by a milder sensory stimulus (prepulse) when presented shortly before the startling stimulus (pulse), a phenomenon termed prepulse inhibition (PPI) (Hoffman & Searle, 1965). PPI can involve any combination of visual, tactile, or auditory stimuli. PPI is believed to entail sensory gating mechanisms as well as direct motor reflex inhibition. An expansive network of brain regions underlies startle, PPI, and modulation of PPI. This network includes nuclei within the pontine reticular system, the superior and inferior colliculi, substantia nigra, basolateral amygdala, hippocampus, thalamus, prefrontal cortex, ventral pallidum, striatum, and ventral tegmental area (Swerdlow *et al.*, 2001). In humans, PPI is believed to be related to an individual's ability to filter incoming sensory information as well as inhibit resultant behavior (Braff *et al.*, 2001; Rabin *et al.*, 2009), and has been shown to be impaired in a number of neuropsychological disorders including schizophrenia and autism (Braff *et al.*, 2001). PPI is a useful experimental technique used to investigate sensorimotor gating mechanisms and test therapeutic treatments in animal models of neurologic disease as well as in clinical populations, most extensively in patients with schizophrenia (Swerdlow *et al.*, 2008).

The aim of this study was to investigate an established rat model of decreased PPI induced by administration of the NMDA antagonist, dizocilpine (Mansbach & Geyer, 1989), and the reversal of this PPI impairment by the histaminergic H1-antagonist, pyrilamine. H1-antagonism is a potential mechanism of the therapeutic effects of the atypical antipsychotic, clozapine, which improves PPI following dizocilpine administration in rats as well as in patients with schizophrenia (Kumari & Sharma, 2002; Levin *et al.*, 2007; Roegge *et al.*, 2007). In the present study we show that chronic pyrilamine administration prevents the PPI impairment induced by chronic dizocilpine administration, an effect that is correlated with a reduction in ligand-binding potential of H1 receptors in the anterior cingulate and an increase in nicotinic receptor α7 subunit binding in the insular cortex. In light of the functional anatomical connectivity of the anterior cingulate and insular cortex, both of which interact extensively with the core PPI network, our findings support the inclusion of both cortical areas in an expanded network capable of regulating sensorimotor gating.

Methods

Animals

Thirty six adult female Sprague-Dawley rats (9 per treatment condition) were used for the studies (obtained from Taconic Labs, Germantown, NY). All protocols for the study were approved by the Institutional Animal Care and Use Committee (IACUC) of Duke University. The rats were housed in groups of three in a temperature-controlled vivarium on a 12 hr/12 hr reverse light-dark cycle with ad-lib access to food and water. They were tested during the dark phase.

Equipment

Acoustic startle reflex amplitude was measured and prepulse inhibition levels were calculated using the Med Associates Startle Reflex System (St. Albans, VT, USA). The equipment included response platforms that were placed in sound attenuating chambers. Each platform was calibrated with a spinner-type calibrator (Med Associates Startle Calibrator). A speaker was placed within the chamber midway along the long axis of the platform. The sound intensity of the speaker in each chamber was calibrated before test sessions (Digital Sound Level Meter, Extech Instruments). Plexiglas cylinders large enough to allow animals to turn around (7.5 cm diameter), were mounted on the platforms. The background white noise was a constant 65 dB.

Behavioral procedure

The test session was conducted in 3 blocks. After the animals were placed in the chambers, there was a 5 min acclimation period before testing began. Block 1 consisted of 6 pulse-only trials, in which a 20 msec, 110 dB white noise stimulus (pulse) was presented. Block 2 had a total of 48 trials: 12 pulse-only trials and 36 prepulse-pulse trials. Within the prepulse-pulse trials, prepulses consisted of 20 msec pure tone noises of one of 3 possible intensities: 68, 71, or 77 dB. The trials were presented in random order with the inter-trial duration ranging from 10–20 seconds. Block 3 had an additional 5 pulse-only trials. Each stimulus had a 2 msec rise/fall time. The null period was 100 msec and the prepulse-to-pulse interval was 100 msec onset to onset. The entire test period lasted approximately 34 mins.

Following testing, the data were analyzed to determine percent PPI for each animal. The mean percent PPI values reported were calculated in the following way:

$$mean\,\%\,PPI = \frac{(mean\ pulse\ only\ startle - mean\ prepulse\ pulse\ startle)}{(mean\ pulse\ only\ startle)}$$

The data from each trial were averaged for each animal, separately for each prepulse intensity, to obtain this measure of percentage inhibition of the acoustic startle responses by the prepulses.

Drug treatment

Dizocilpine (MK-801, Sigma-Aldrich, St. Louis, MO) (0.15 mg/kg/day) was administered via 2ML4 osmotic minipumps for 4 weeks. The Alzet osmotic minipumps (Durect, Cupertino, CA) were implanted subcutaneously in between the scapulae on the back, in a sterile surgical procedure following manufacturer guidelines. This was covered under our approved IACUC protocol. Control animals were administered vehicle consisting of sterile 0.9% saline (Hospira, Lake Forest, IL) during this period, and pyrilamine (Sigma-Aldrich, St. Louis, MO) (50 mg/kg/day) was similarly administered with (n = 9) or without (n = 9) dizocilpine. Every week, the rats were tested for PPI, and before the final testing session in week 4, pharmacological treatments were withdrawn for one day.

Quantitative receptor autoradiography

Following behavioral analyses, the rats were anesthetized with Euthasol (100 mg/kg, Virbac, Inc., Fort Worth, TX, USA) and then sacrificed by exsanguination. Brains were immediately removed and stored at -80°C. To prepare histological sections, each brain was sliced coronally at 20 µm thickness using a cryostat (Bright Instruments, Cambridgeshire, UK) and sections were mounted on gelatin-subbed slides (precleaned Superfrost Fisherbrand slides treated with 0.5% gelatin and 0.05% chromium potassium sulphate). All

radioligands were purchased from American Radiolabeled Chemicals (Saint Louis, MO). Specific binding of the α7 subunit of the acetylcholine receptor was determined using [125I]α-bungarotoxin (5 nM, 147 Ci/mmol), and specific binding of the histamine H1 receptor was determined using [3H]pyrilamine (5 nM, 20 Ci/mmol). Radioligands were diluted in a chilled buffer containing 50 nM Tris-HCl and 1 mg/ml BSA, pH 7.4 and the entire radioligand binding protocol was performed on ice to limit nonspecific binding. Slides were first pre-incubated in buffer for 1 hr, after which they were incubated with the appropriate radioligand solution for 1 hr. Non-specific binding was assessed by co-incubation with L-nicotine (4 μM, Acros Organics, NJ) or nonradioactive pyrilamine (2 μM, MP Biomedicals, CA) for the α7 subunit and H1-receptor, respectively. Following incubation in radioligand solution, the slides were washed for 15 min in three changes of chilled buffer, and then subsequently dried with blown air at room temperature. Sections were then exposed to film (Kodak, Hyperfilm) for 22 days to visualize α7 subunit labeling and approximately 6 weeks to visualize pyrilamine labeling. Films were scanned (Epson, Perfection V750 Pro) and analyzed densitometrically using Image-J (Schneider *et al.*, 2012). Each film was exposed to the appropriate set of radioactive standards, and densitometric data were converted to fmol/mg tissue equivalent.

Radioligand binding was quantified within a distributed network of brain regions (Figure 1) previously implicated in regulating PPI of startle (Swerdlow *et al.*, 2001). The regions we investigated within this network included the inferior colliculus, amygdala, and hippocampus. Within the amygdaloid complex, we sampled within specific groups of nuclei: the basolateral group, the cortical group, and the centromedial group (Sah *et al.*, 2003), and within the hippocampal complex, we sampled CA1-3 of the hippocampus proper, as well as the dentate gyrus. Additionally, we qualified radioligand binding in the anterior cingulate and insular cortex, both of which are known to interact with the PPI network and have been preliminarily implicated in fMRI investigations of PPI in humans (Campbell *et al.*, 2007; Kumari *et al.*, 2007). A stereotaxic atlas (Paxinos, 2005) was used to confirm that each brain region was sampled consistently, and data from each brain hemisphere were averaged prior to statistical analysis.

Statistical analysis

Analysis of Variance (ANOVA) tests were used to assess differences between group means on the basis of pharmacologic treatment. Post-hoc comparisons were performed using Tukey's HSD tests. Furthermore, to assess the overall effects of pyrilamine treatment, rat data were clustered into two groups (with and without pyrilamine treatment) and independent samples t-tests were performed. The appropriate t-test was chosen based on the results of Levene's test of equality of variances between each group. For brain regions that demonstrated significant differences in radioligand binding between groups, Pearson correlations were performed to test the association between radioligand binding and PPI. All statistical tests were two-sided, with an alpha level of 0.05. Confidence intervals were set at 95% for all comparisons. Statistical computations were performed with SPSS Version 19 (IBM, Armonk, NY, USA).

Results

Pyrilamine treatment reverses PPI impairment in dizocilpine-treated rats

Although prepulse intensity did not significantly affect inhibition of the startle response, we did observe a trend towards increased PPI with increased prepulse intensity (Figure 2, Dataset 1). For trials using the 77 dB prepulse, the highest intensity employed, ANOVA

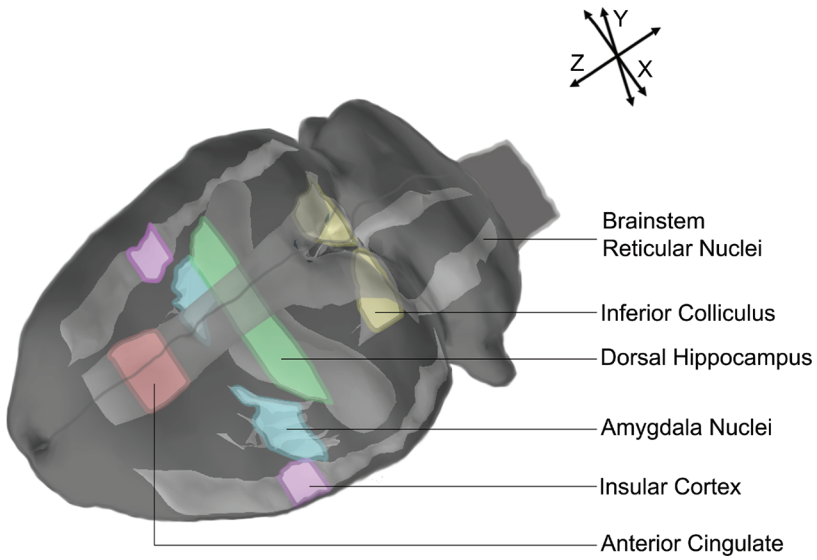

Figure 1. Diagram of brain regions investigated. Each of the regions investigated with autoradiography are shown in light grey and are labeled. The colored highlighted portions of each region demonstrate the precise location within which autoradiographic analyses were performed in this study. This figure was generated from images acquired in a 3-D stereotaxic rat brain atlas software (Brain Navigator release 2.0, 2009).

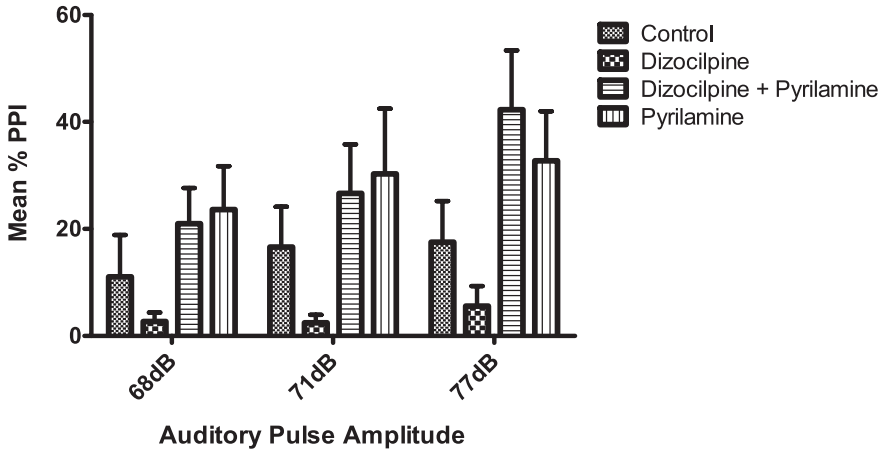

Figure 2. Pyrilamine treatment attenuates dizocilpine-induced reduction in PPI. At each prepulse decibel (dB) level employed, the general pattern of pharmacologic manipulation of PPI was maintained: pyrilamine treatment increased PPI and attenuated dizocilpine-induced PPI impairment. Furthermore, in agreement with prior reports, increased prepulse intensity was associated with stronger prepulse inhibition. Error bars represent standard error of the mean.

demonstrated a significant effect of pharmacologic treatment ($p = 0.026$), with pyrilamine administration significantly attenuating the dizocilpine-induced PPI impairment ($p = 0.021$).

Pyrilamine treatment decreases H1-receptor binding in the anterior cingulate, an effect correlated with PPI improvement

Within the anterior cingulate, pyrilamine treatment induced a 37 ± 19% decrease in H1-receptor binding that was nearly significant ($p = 0.058$) (Figure 3, Dataset 2). This change correlated with PPI such that decreased H1-receptor binding was associated with increased mean PPI at all three prepulse intensities (Figure 4, $R^2 = -0.15$, $p = 0.05$). Note as demonstrated in Figure 4, Figure 6, and Figure 7 that some rats (irrespective of pharmacologic treatment) did not demonstrate the PPI phenomenon: this is a common occurrence in PPI studies and reflects inter-individual differences.

Acetylcholine receptors in the insular cortex are likely involved in pyrilamine-induced PPI improvement

Within the insular cortex, pyrilamine treatment induced a 9 ± 3% increase in α7 acetylcholine (ACh)-receptor binding ($p = 0.002$) (Figure 5, Dataset 2). Increased α7 ACh-receptor binding correlated with increased mean PPI at all three prepulse intensities (Figure 6, $R^2 = 0.24$, $p = 0.004$).

Multiple mechanisms of pyrilamine-induced PPI improvement

The results from this study imply that pyrilamine modulates PPI of the startle response through diverse mechanisms that are regionally discrete. In the anterior cingulate, α7 ACh-receptor binding was

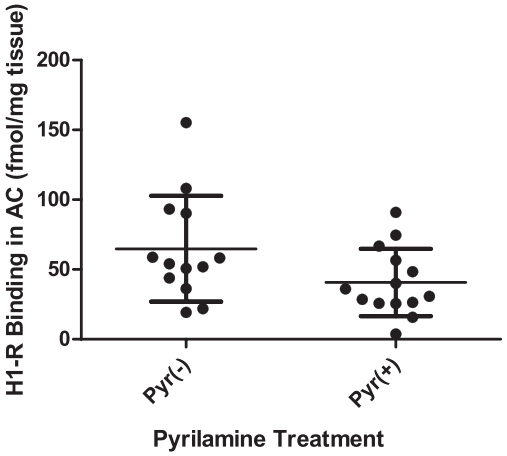

Figure 3. Pyrilamine treatment results in decreased H1 receptor binding in the anterior cingulate. An overall effect of pyrilamine treatment was a reduction in H1-receptor binding in the anterior cingulate (AC). This was a nearly significant trend that will require further investigation to confirm ($p = 0.058$).

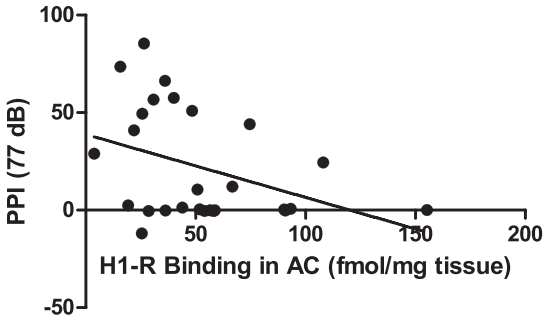

Figure 4. H1 receptor binding in the anterior cingulate correlated with PPI. There was a negative correlation between H1 receptor binding in the anterior cingulate (AC) and PPI, such that lower H1 binding in the AC was associated with higher PPI. Data are displayed for the 77 dB prepulse trials for which PPI was greatest.

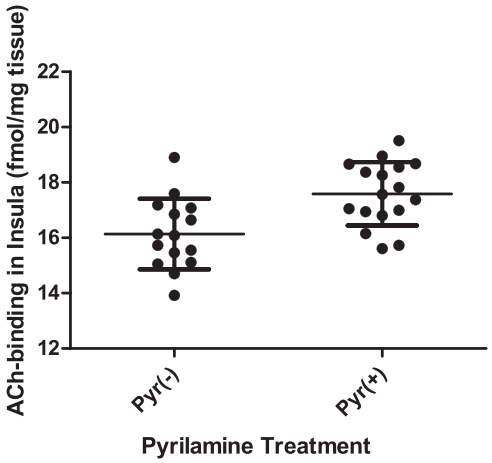

Figure 5. Pyrilamine treatment results in increased α7-nicotinic receptor binding in the insula. An overall effect of pyrilamine treatment was an increase in α7-nicotinic receptor binding in the insular cortex (p = 0.002).

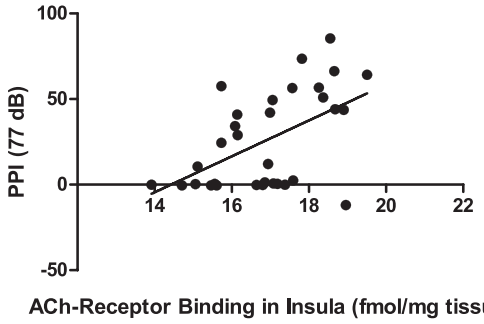

Figure 6. α7 receptor binding in the insula cortex correlated with PPI. We observed a positive correlation between α7-nicotinic receptor binding in the insular cortex and PPI, such that greater α7 binding in the insula was associated with higher PPI. Data are displayed for the 77 dB prepulse trials for which PPI was greatest.

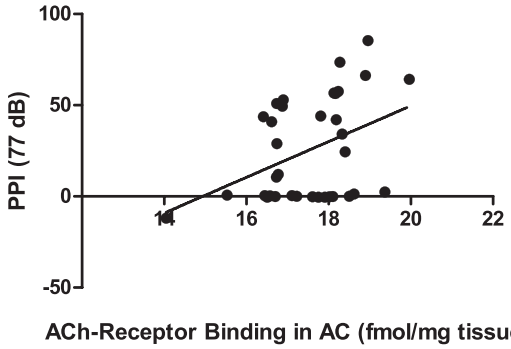

Figure 7. α7 receptor binding in the anterior cingulate correlated with PPI. Although pyrilamine treatment did not affect α7 receptor binding in the anterior cingulate (AC), we did observe a positive correlation between α7 receptor binding in this region and PPI. This indicates that α7 receptors in the AC may contribute to PPI independently of H1 receptor-mediated signaling.

positively correlated with mean PPI (Figure 7, $R^2 = 0.14$, p = 0.027); however, α7 ACh-receptor binding in this region was not significantly affected by pyrilamine treatment (p = 0.507, Dataset 2). Likewise, pyrilamine treatment did not affect H1-receptor binding in the insular cortex (p = 0.237), and H1-receptor binding in the insula was not associated with mean PPI (p = 0.702). In the other brain regions investigated (inferior colliculus, amygdala, and dorsal hippocampus), neither H1-receptor binding nor α7 ACh-receptor binding was associated with PPI levels.

Prepulse inhibition of the startle response and radioligand binding assays

2 Data Files

http://dx.doi.org/10.6084/m9.figshare.1060215

Discussion

Sensorimotor gating impairment has been associated with a wide variety of neurological and psychiatric disorders. It has been observed in patients with sensory processing disorder (Davies *et al.*, 2009), Parkinson's disease (Nakashima *et al.*, 1993), schizophrenia (Braff *et al.*, 2001), non-epileptic seizures (Pouretemad *et al.*, 1998), Tourette's syndrome and ADHD (Castellanos *et al.*, 1996), nocturnal enuresis (Ornitz *et al.*, 1992), blepharospasm (Gómez-Wong *et al.*, 1998), obsessive-compulsive disorder (Swerdlow *et al.*, 1993; Hoenig *et al.*, 2005), panic disorder (Ludewig *et al.*, 2002), bipolar disorder (Perry *et al.*, 2001; Rich *et al.*, 2005), Huntington's disease (Swerdlow *et al.*, 1995), Fragile X syndrome (Hessl *et al.*, 2009), and autism (Perry *et al.*, 2007). In humans, sensory gating measures such as PPI have been found to correlate with a growing list of behavioral symptoms, including neuroticism, disinhibition on the go/no-go task, premonitory urges in Tourette's syndrome, high trait anxiety in panic disorder, restricted/repetitive behavior in autism, and in schizophrenia: positive and negative symptoms, semantic priming abnormalities, perseveration, information-processing deficits, thought disorder, distractibility, and violent behavior (Perry & Braff, 1994; Karper *et al.*, 1996; Vinogradov *et al.*, 1996; Braff *et al.*, 1999; Corr *et al.*, 2002; Ludewig *et al.*, 2002; Perry *et al.*, 2007; Rabin *et al.*, 2009; Yadon *et al.*, 2009). It is important to investigate the neural bases that underlie dysfunctions of sensory gating in order to develop effective therapeutic treatments.

The present study employed a PPI paradigm in which an auditory startle stimulus was immediately preceded by a milder auditory prepulse expected to induce significant gating of the startle response. Chronic administration of the NMDA antagonist, dizocilpine, caused a considerable deficit in sensorimotor gating, demonstrated by the low levels of PPI of the startle response in dizocilpine-treated rats (mean PPI of 5.6%). This deficit was entirely reversed by co-administration of the selective histaminergic H1-receptor antagonist, pyrilamine (Figure 2).

Dizocilpine disruption of PPI has been previously used to model sensorimotor gating impairments (Mansbach & Geyer, 1989; Geyer *et al.*, 2001). The predictive validity of this model is supported by the fact that the atypical antipsychotic, clozapine, reverses PPI impairment in this model (Levin *et al.*, 2007; Lim *et al.*, 2012) as it does similarly in humans (Nagamoto *et al.*, 1996; Adler *et al.*,

1998). Here we show that chronic pyrilamine treatment mimics the effect of clozapine, reversing PPI impairment in the dizocilpine-treated rat. As clozapine has been shown to saturate the brain's H1-receptors at therapeutic concentrations (Humbert-Claude *et al.*, 2012), this may provide an important mechanism of clozapine's therapeutic action.

In the mammalian brain, histaminergic fibers originating in the tuberomammillary nucleus of the posterior hypothalamus regulate the response to noxious stimuli (Itoh *et al.*, 1989) and infection (Saper *et al.*, 2012), and also regulate the excitability of arousal circuits (Tasaka *et al.*, 1989). One mechanism of histamine's effects on arousal is through regulation of cholinergic transmission in the nucleus basalis of Myenert in the basal forebrain (Bacciottini *et al.*, 2001; Dringenberg & Kuo, 2003). Histamine's cholinergic effects have been shown to differ regionally in the brain: in the frontoparietal cortex, local histamine administration inhibited acetylcholine release by 50% (Blandina *et al.*, 1996), whereas histamine application to the nucleus basalis has been shown to double cholinergic output to the cortex in rats (Cecchi *et al.*, 1998). The complicated interactions between histaminergic signaling and cholinergic tone throughout the brain awaits further elucidation; however, it has been shown that tuberomammillary lesions or systemic administration of the H1 antagonists, chlorpheniramine and pyrilamine, are similarly capable of significantly increasing cortical acetylcholine dose-dependently (Dringenberg *et al.*, 1998). Further, members of our group have recently shown that pyrilamine treatment reduces nicotine self-administration in rats (Levin *et al.*, 2011). So it is believed that systemic H1 antagonists modify behavior in part by increasing cortical cholinergic transmission.

The role of histaminergic signaling in PPI has not been extensively studied, but of the disorders investigated to date that are associated with sensorimotor gating abnormalities, all have displayed increased histaminergic neurotransmission. These include: Parkinson's disease (Rinne *et al.*, 2002), Tourette's syndrome (Fernandez *et al.*, 2012), bipolar disorder (Jin *et al.*, 2009), Huntington's disease (van Wamelen *et al.*, 2011), and schizophrenia (Ito, 2004; Arrang, 2007). In Alzheimer's dementia, interestingly, researchers found significantly decreased histamine levels in the frontal and temporal cortices (Mazurkiewicz-Kwilecki & Nsonwah, 1989), and normal PPI (Hejl *et al.*, 2004).

Prior work by members of our group demonstrated the ability of a single dose of pyrilamine to attenuate PPI impairment in rats acutely administered dizocilpine or amphetamine (Roegge *et al.*, 2007; Larrauri & Levin, 2010). The current study was designed to further explore this phenomenon pharmacologically in rats by chronically administering dizocilpine and/or pyrilamine, a scenario more analogous to long-term therapeutic enhancement of sensorimotor gating in humans. Our aim was to determine which components of the distributed PPI network were impacted by pyrilamine treatment to improve PPI.

Pyrilamine treatment resulted in decreased H1-receptor binding in the anterior cingulate, which was correlated with PPI improvement (Figure 3 and Figure 4). Functional involvement of the anterior cingulate in PPI has been previously demonstrated through lesion as well as fMRI and PET studies (Hazlett *et al.*, 1998; Yee, 2000; Goldman *et al.*, 2006; Campbell *et al.*, 2007; Neuner *et al.*, 2010), and this region is known to express a high concentration of H1-receptors (Tagawa *et al.*, 2001). As a site of limbic and cortical integration, the anterior cingulate modulates conditioned fear responses and arousal (Hamner *et al.*, 1999). The anterior cingulate has been shown to send efferent projections to the amygdala (Wang *et al.*, 2009), a wide distribution of thalamic nuclei (Fujii, 1983), substantia nigra (Beckstead, 1979), nucleus accumbens (Sesack *et al.*, 1989), globus pallidus (Beckstead, 1979), and superior colliculus (Sesack *et al.*, 1989), all regions implicated in regulating PPI of the startle response (Yamada *et al.*, 1998; Fendt *et al.*, 2001; Hazlett *et al.*, 2001; Takahashi *et al.*, 2007; Forcelli *et al.*, 2012). Efferents of the anterior cingulate have even been traced to the giant neurons of the caudal pontine reticular nucleus, which display prepulse-inhibited membrane potential and are believed to be an integral component of the acoustic startle response (Sesack *et al.*, 1989; Lingenhöhl & Friauf, 1994). Similarly, anterior cingulate efferents were traced to the adjacent pedunculopontine tegmental nucleus (Sesack *et al.*, 1989), which is an established component of the core brainstem PPI circuitry that directly modulates the pontine reticular giant neurons through cholinergic innervation (Fendt *et al.*, 2001). We have shown that α7 ACh-receptor binding in the anterior cingulate is positively correlated with PPI (Figure 7), so it is possible that cholinergic signaling in this region plays a role in PPI. Indeed, nicotine has been shown to increase activity in the anterior cingulate while improving PPI (Postma *et al.*, 2006). However, we found no effect of pyrilamine on the α7 ACh-receptor binding in the anterior cingulate, implicating an alternate mechanism of pyrilamine enhancement of PPI in this brain region.

Contrary to the anterior cingulate, the insular cortex displayed a significant increase in α7 ACh-receptor binding in pyrilamine-treated rats that was positively correlated with PPI (Figure 5 and Figure 6). However, there was no change in H1-receptor binding in the insula of rats that were treated with pyrilamine. Because H1 antagonists have been shown to be ineffective in regulating insular cortical excitability when directly applied to this region (Takei *et al.*, 2012), pyrilamine treatment may modulate insular acetylcholine signaling indirectly. The insular cortex contains a high concentration of nicotinic receptors, and has shown increased activation following nicotine administration in patients with schizophrenia whose PPI was improved by nicotine (Postma *et al.*, 2006). Agonism of α7 ACh-receptors has been demonstrated to improve sensory gating in patients with schizophrenia (Martin & Freedman, 2007), and has proven beneficial in animal models of not only gating impairments (Cilia *et al.*, 2005; Hajós *et al.*, 2005; Thomsen *et al.*, 2009) but also positive and negative symptoms of schizophrenia (Hauser *et al.*, 2009). Therefore, it is likely that pyrilamine-induced increases in insular α7 ACh-receptor expression contribute to the observed improvement in PPI.

Due to its anatomic connections, the insular cortex is believed to integrate the processing of autonomic responses with that of ongoing behavioral plans and emotional states (Allen *et al.*, 1991; Gu *et al.*, 2013). The insula is anatomically poised to influence top-down processing of sensorimotor gating, with both direct as well as indirect connections to the pontine reticular startle circuit (Wiesendanger

& Wiesendanger, 1982). The insula send largely reciprocal projections to the amygdala and mediodorsal nucleus of the thalamus (Shi & Cassell, 1998), substantia nigra, raphe nucleus, ventral pallidum, and ventral striatum (Reep & Winans, 1982), all of which have been associated with regulation of PPI (Young *et al.*, 1995; Fendt *et al.*, 2001; Adams *et al.*, 2008; Baldan *et al.*, 2011; Forcelli *et al.*, 2012). The insula have furthermore been shown to be involved in distinguishing successive stimuli presented with a short interstimulus time interval as is required to elicit PPI (Kosillo & Smith, 2010).

Although the anterior cingulate and insular cortex have been shown to be highly interconnected functionally (Di Martino *et al.*, 2009; Medford & Critchley, 2010; Cauda *et al.*, 2011), there was no correlation between the changes we report in pyrilamine binding in the anterior cingulate and α-bungarotoxin binding in the insular cortex. The anterior cingulate and insular cortex both send projections to regions that are known to directly modulate the startle response through interaction with the pontine reticular nuclei responsible for this reflex. It is therefore possible that systemic pyrilamine treatment modified the activity of the anterior cingulate and insular cortex independently to improve PPI, although further research is necessary to understand the interaction between anterior cingulate and insular cortical networks in modulating startle inhibition. This study demonstrates the wide distribution of networks capable of influencing PPI of startle, supporting the importance of the PPI measure as a tool to analyze interactions between multiple hierarchies of neuronal processing in disparate brain regions, particularly in pathological states. It will be important to further investigate effects of pharmacological treatments on PPI in humans as well as animal models of disease to elucidate the neuronal machinery underlying sensory filtering and behavioral inhibition. The involvement of H1 receptors in PPI has not only been demonstrated in pharmacologically induced PPI disruption models, but also in models of developmental disturbance that show PPI impairment. In a mouse model of isolation rearing, pyrilamine treatment or H1 receptor knockout were capable of preventing PPI impairment (Dai *et al.*, 2005) similarly to clozapine (Möller *et al.*, 2011). Along these lines, further research into the histaminergic regulation of PPI is warranted, as H1-antagonists have demonstrated a low incidence of side effects in clinical trials (Pearlman *et al.*, 1997; Lankford *et al.*, 2012). A recent initial clinical study has shown that the antihistamine meclizine significantly improves PPI in people who have less than typical PPI and exaggerated startle response (Larrauri *et al.*, 2014). H1 antagonists may therefore prove useful in reversing sensory gating dysfunctions. Further evaluation should determine the possible efficacy and side effects of this novel line of treatment.

Data availability

figshare: Prepulse inhibition of the startle response and radioligand binding assays, doi: 10.6084/m9.figshare.1060215 (Skefos *et al.*, 2014)

Author contributions

EDL and MLB conceived the study. EDL, MLB and JS designed the experiments. JL and EK carried out the animal surgery, behavioral testing, and dissection. JS, MG, AM, and GP carried out the tissue preparation, radioligand binding protocols, and quantification. JS carried out the data analyses and prepared the first draft of the manuscript. EDL, MLB, JL, and JS revised the manuscript. All authors provided input in the design of this study and the reporting of the findings.

Competing interests

No competing interests were disclosed.

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This is an interesting attempt to link histaminergic changes in PPI during H1 receptor blockade with pyrilamine in the presence of the NMDA receptor antagonist dizocilpine (MK-801) with changes in H1 binding measured by autoradiography. However, I was left with several questions regarding the study population and presentation of the data:

1. The study was conducted on 36 female rats, with no rationale provided for the exclusion of males. It would be helpful to consider in the discussion how this choice may impact PPI changes in light of steroid receptor interactions with both H1 and a7-nicotinic receptors.

2. The association between decreased H1 binding and increased PPI is presented in the results as "R^2 = -0.15", which is not possible, so I assume that refers to the Pearson's R value. Thus the correlation was very weak, and although they report a significant result (p=0.05), that is hardly a strong effect. Examination of figure 4 suggests that the population may have divided into responders and non-responders, at least for the PPI measured at 77dB. Approximately 9 of the data points in figure 4 show negligible PPI even at the lowest measured H1 binding, which suggests that there may be other factors affecting this relationship. Cluster analysis of the data might be revealing in this context.

3. No rationale is provided for testing only a single dose of pyrilamine, which is somewhat problematic given the very small difference between pyrilamine (+) and (-) animals in figure 3, which compares H1 binding in the anterior cingulate between treated and untreated animals. The description of this result as a "nearly significant trend" (p=0.058) is also unconvincing, especially as there is a possible outlier in the pyr (-) group. A Dixon test or similar approach could be applied to determine if this is a true outlier, in which case there would be virtually no difference between groups.

4. It would be helpful to include in the discussion some consideration of the specificity of pyrilamine for the H1 receptor, and the possible consequences of the dosage applied on other receptor subtypes or classes. A number of recent reports are available which discuss the interactions of pyrilamine with nicotinic receptors in other contexts (e.g. Sadek *et al.*, 2015; Kim *et al.*, 2014), and these should be cited and discussed with respect to the present findings.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Referee Response (*Member of the F1000 Faculty*) 11 Feb 2015

David Reser, Department of Physiology, Monash University, Australia

The more I look at this, the more uncomfortable I am with the choice of analysis methods applied to the comparison of receptor binding with PPI change. In figures 6 and 7, nearly all of the subjects with non-zero PPI have y-axis values above the line of correlation, due to the absence of PPI in all of the other subjects. In a sense, virtually all of the data points in this correlation analysis are "residuals", located well away from what should approximate a first-order line of best fit through the population. The correlation measures are thus biased to the point of uselessness, since they really reflect the middle values between populations of apparent responders and non-responders. I strongly recommend that the data in these figures, along with the data from fig. 4 (as called out in the review above) be re-analyzed, possibly with the assistance of a statistician, as these populations are quite likely clustered, and therefore not suitable for a simple correlation analysis.

Competing Interests: No competing interests were disclosed.

Referee Report 21 July 2014

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Stan Leung
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Skefos *et al.* reported that administration of H1 receptor antagonist pyrilamine with NMDA receptor antagonist MK-801 (daily for 4 weeks) reversed the impairment of auditory prepulse inhibition (PPI) caused by dizocilpine (MK-801) administration alone. Pyrilamine treatment resulted in an increase in α7 nicotinic receptor binding in the insular cortex, which also correlated with PPI improvement.

A change in α7 nicotinic receptor binding in the insular cortex after pyrilamine administration, in correlation with PPI improvement, is a novel result. α7 nicotinic receptor binding in the anterior cingulate cortex also correlated with PPI, but did not change with pyrilamine treatment. The participation of histamine in cholinergically mediated arousal forebrain circuits is known for some time and nicotinic agonism, and possibly an increase in α7 nicotinic receptor binding in the insular cortex, may improve PPI. However, how administration of an H1 antagonist resulted in increased α7 nicotinic receptor binding is not known. The authors' inference of a "wide distribution of networks capable of influencing PPI of startle" is likely correct. While a direct participation of insular cortex in PPI is not excluded, correlation of binding with PPI may suggest two separate and parallel events after pyrilamine treatment, without cause and effect relation.

The title is appropriate. The abstract reads well, but should state **chronic** pyrilamine treatment. The paper

is generally well written. However, some methods and results, and sample sizes (n) should be presented more clearly or explicitly before indexing.

The methods included testing PPI every week, and the result presented in Fig. 2 was apparently for the final PPI test at 4 weeks. Were similar results observed for the PPI tests at earlier times? Did the groups differ in their startle response (without prepulse)? To be clear, the Results section should state “pyrilamine **with dizocilpine**” administration (not just pyrilamine) “significantly attenuating the dizocilpine-induced PPI impairment.” Did pyrilamine alone in Fig. 2 significantly alter PPI as compared to saline alone? The equation for PPI should have x100% on the right side.

The Results and figures/legends did not state the sample sizes, and in some cases, the inclusion criteria were not clear. There were apparently 4 groups as indicated in Fig. 1 (control, saline administered; pyrilamine alone; MK-801 alone; MK-801+ pyrilamine). In the Methods, the number of rats (n=9) was only stated for the last two groups. (The data sets do clarify that the other two groups also had n=9). Do data points of “no pyrilamine” Pyr(-) in Fig. 3 correspond to the MK-801 group without pyrilamine or also include the saline control group? Or does the Pyr(+) group include data with and without MK-801? Did Pyr have the same effect with and without MK-801? Similar questions can be asked for other figures, and the authors should be explicit as to which groups were included. Currently, Fig. 3 appears to have 13-14 points for each of the Pyr (-) and Pyr (+) groups, and ~24 points for the correlation in Fig. 4 [Fig. 5: 15 & 17 points, and Fig. 6, 30 points]. These “n” are unclear to the reader, and should be stated in the figure or figure legend. If the authors had used more than one point for each rat, this has to be explained. Statistics should include degrees of freedom (df) or sample size (n); e.g., 37 ± 19% (give n), and R^2 statistic should include df.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.