In search of lost time: cognitive and arginine metabolic correlates of temporal dysfunction in the MIA rat model of schizophrenia risk

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Abnormal temporal perception is a hallmark characteristic of schizophrenia associated with cognitive impairment, however the relationship between these functions is yet to be characterised within translational models. Using the maternal immune activation (MIA) rat model, this study investigated the contribution of sustained attention and working memory capacity to temporal perception impairments via operant paradigms. In addition, we also investigated the involvement of L-arginine metabolites in timing and cognition via high performance liquid chromatography and liquid chromatography/mass spectrometry. Principally, we identified that underestimation of interval durations (2-8 s) in MIA rats was related to attentional capacity. MIA rats were found to exhibit impaired working memory maintenance, however this was not related to temporal perception. In addition, we identified evidence of MIA impacting PFC L-arginine metabolites, L-citrulline and putrescine, which both correlated with working memory maintenance impairments. MIA also appeared to produce discrete differences in glutamate levels depending on whether inflammation was incited early or late in gestation (gestation day 10/18). Following late gestation MIA exposure, higher glutamate levels in PFC corresponded with poorer sustained attention capacity. These findings represent the first direct identification of a timing-attention relationship within rodents, and provide clues regarding the potential involvement of elevated dopamine in timing-cognition pathology in schizophrenia. Moreover, we present preliminary evidence that changes in L-arginine metabolism have functional consequences for cognition. These outcomes commend the MIA rat model as a tool for potential future investigations exploring the biological instantiation of timing deficits.

Abbreviations

HPLC = high performance liquid chromatography

LC/MS = liquid chromatography mass spectrometry

MIA = maternal immune activation

PFC = prefrontal cortex

vITI = variable inter trial interval

HE = head entry

CR = choice response

SA = sustained attention

WM = working memory

Poly IC = polyinosinic:polycytidylic acid

PSE = point of subjective equality

PLM = pseudo-logistic model

GD = gestation day

Schizophrenia is a complex neurodevelopmental disorder which manifests as impairment within cognitive, emotional, and behavioural domains. Despite having low prevalence (0.28%), schizophrenia is ranked as the 12th most disabling disorder worldwide; population growth and increased life span in conjunction with poor clinical efficacy are purported to be driving increases in disease burden (Charlson et al., 2018; Insel, 2010; Vos et al., 2017). Current pharmacotherapeutic options fail to fully attenuate symptomology owing to their targeting of common mechanisms of action (namely dopamine D2 receptor blockade; McCutcheon et al., 2020; Miyamoto et al., 2005). As such, present focus within this field is on elucidating the pathophysiology instantiating schizophrenia with the goal of optimising treatment approaches.

Abnormal perception of time is commonly documented yet remains an understudied symptom of schizophrenia (Gómez et al., 2014). Abnormal timing has been linked to learning deficits, as well as features of psychosis including hallucinations and delusions (Gómez et al., 2014; Roy et al., 2012). In the case of prospective timing of interval durations (durations in the range of seconds to minutes), individuals with schizophrenia exhibit increased perceptual variability (Thoenes & Oberfeld, 2017). Changes in temporal accuracy, however, are task dependent, reflecting task specific recruitment of interrelated processes such as attention and memory (Thoenes & Oberfeld, 2017). The temporal bisection task, for example, is commonly used to index temporal impairment in schizophrenia. Originally designed by Church & Deluty (1977), the temporal bisection task trains participants to discriminate between two anchor cues of particular durations, one ‘short’ and one ‘long’. Following this, a range of cue durations intermediate to the two anchor cues are presented, which participants subsequently categorize as being subjectively more alike either the short or long anchor cue durations. Fitting a psychometric function to the data yields accuracy and precision parameters. A meta-analysis of temporal bisection studies using cues in the millisecond to second range found that individuals with schizophrenia underestimated elapsed durations compared to controls, corresponding to a subjective quickening of temporal passages (Thoenes & Oberfeld, 2017).

The biological instantiation of timing is unknown, however midbrain dopamine (DA) is considered to play a critical role in determining the pace of temporal perception. Administration of DA agonists (cocaine, methamphetamine) or neuroleptics (haloperidol, promazine) in humans and rats, as well as optogenetic activation or inhibition of rodent midbrain DAergic neurons, has been found to respectively increase or decrease temporal pace (Meck, 1986; Papageorgiou et al., 2013; Soares et al., 2016). Pathological mesolimbic DAergic function has been implicated in a multitude of disorders which present with temporal abnormalities, including Parkinson’s disease, attention-deficit hyperactivity disorder (ADHD) and schizophrenia (Allman & Meck, 2012). In the case of schizophrenia, a meta-analysis of in vivo PET (positron emission tomography) and SPECT (single photon emission computed tomography) imaging found that presynaptic DA availability and release

was elevated in the striatum of treatment-naïve individuals relative to controls (Howes et al., 2015; Howes et al., 2012). The pathology informing temporal abnormalities in schizophrenia may be more broad however, as a meta-analysis of functional imaging data found that both cortical and subcortical activation of areas involved in timing was lesser in the right hemisphere, with authors theorising that reduced hemispheric synchronicity may mediate abnormal timing (Ortuño et al., 2011).

A key challenge in understanding the biology of timing is that there is considerable overlap in the areas implicated in healthy timing and in cognitively demanding tasks (Alústiza et al., 2017). For instance, the recruitment of basal ganglia, thalamus and substantia nigra in timing tasks (Buhusi & Meck, 2005) has since been accounted for by working memory involvement (Wiener et al., 2010). In addition, research indicates that the likelihood of a region being activated during timing is dependent on the extent of cognitive demand (Gómez et al., 2014). Considering that cognitive impairment is a hallmark feature of schizophrenia (Insel, 2010) the extent to which it may be implicated in temporal perception abnormalities is unclear. Independent cognition and working memory measures have been correlated with schizophrenia-mediated temporal impairment (Lee et al., 2009; Roy et al., 2012), while individuals with schizophrenia have been found to display poorer perceptual accuracy during timing tasks which engaged sustained attention and working memory processing (de Montalembert et al., 2016; Tracy et al., 1998). The majority of findings in this area suggest that the severity of temporal disturbance mirrors disease acuteness (Lee et al., 2009). Higher scores in the Positive and Negative Syndrome Scale (PANSS) and in the Brief Psychiatric Rating Scale (BPRS) have been correlated with poorer performance in timing tasks (Papageorgiou et al., 2013).

Translational models have been valuable in elucidating the etiopathogenesis of schizophrenia. The maternal immune activation (MIA) model, for instance, indexes the neurodevelopmental sequelae of prenatal exposure to inflammation, an established risk factor for schizophrenia (Estes & McAllister, 2016). Murine MIA models are well established, recapitulating a range of neuro-behavioural disease characteristics, including altered learning, attention, memory and timing as well as DA dysfunction, (Deane et al., 2017; Murray et al., 2017; Piontkewitz et al., 2012; Wolff et al., 2011) however the shared bases of these functions remain unexplored. MIA has also been found to impair metabolism of arginine a versatile amino acid previously implicated in the pathogenesis of schizophrenia (Jing et al., 2013; Pérez-Neri et al., 2006), although the functional implications of this remain largely unexplored.

The present study expands on previous work from our group, which identified abnormal perception of time in MIA rats (Deane et al., 2017). Using an operant environment, including the temporal bisection task, we aim to investigate whether abnormal timing and cognitive deficits in the MIA model are functionally interrelated, indicating a common pathological basis. In addition, we aim to understand

whether components of the arginine metabolic pathway in the prefrontal cortex (PFC) may be involved in assessed behaviours.

METHODS

All procedures and experiments were conducted in accordance with the ethical guidelines of the University of Otago Ethics Committee.

Animals. Previous work using the MIA mouse model has identified that MIA exposure during early or late gestation produces time specific profile of disease (Meyer & Feldon, 2012), however there is a paucity of research investigating this effect in rats. Here we use gestation day (GD) 10 and 18 to index the early and late effects of MIA.

Under isoflurane anaesthesia, 20 pregnant 3 month old Sprague Dawley dams received injections of either polyinosinic-polycytidylic acid (poly IC; 4mg/kg dissolved in saline) or a saline vehicle on either GD10 or 18. Two male offspring per dam were retained and housed in pairs within litter groups. Rats were maintained on a 12 hour light/dark cycle (6am to 6pm). All behavioural investigations were conducted during the light phase. A total of 40 animals were used in the following experiments (10 MIA10; 10 CON10; 10 MIA18; 10 CON18). Experimental involvement commenced at three months of age. Rats had *ad libitum* access to water, and were maintained at 85% of their free feeding weight.

Operant tasks. Operant sessions occurred once per day, seven days per week. See Deane et al., (2017) for operant specifications and lever press training methods.

Bisection. The temporal bisection task (Church & Deluty, 1977) was used to assess temporal perception. During the training phase, animals are reinforced for discriminating between two auditory anchor cues (2s 'short' and 8s 'long'), responding via correct selection of one of bilateral levers. During the testing phase, unreinforced intermediate duration probes (2.6, 3.2, 4, 5, 6.4s) are also presented, which animals categorise as either short or long by pressing the levers associated with the 2 and 8 s anchor samples. Plotting the proportion of long responses as a function of sample duration generally produces an increasing sigmoidal function from which quantitative modelling can be used to yield measures of multiple separable components of temporal processing (see Data Analysis).

Bisection training proceeded in several phases. The criterion to proceed to the next choice training phase was defined as 80% correct choice. A total of 70 trials were presented in each session, with variable inter trial intervals (vITIs; μ=45s). Tone stimuli were 30dB. Training details are available in supplementary materials (S1.1).

Cognition. To investigate cognitive abilities, rats were trained on a two choice visual discrimination paradigm. In the presence of the house-light, animals were trained to discriminate between two lateralised LED light stimuli, responding ipsilaterally on the levers located beneath. Following this, the duration of the light stimuli was systematically reduced (2, 1, 0.5, 0.25 s) in order to increase the demand placed upon attentional resources. Rats were then retrained to a standard 2s light duration,

before working memory maintenance was assessed by manipulating the delay between stimuli offset and lever presentation (2, 4, 8, 16 s; Kahn et al., 2012). Plotting the proportion of correct responses as a function of a) decreasing stimulus duration, and b) increasing response delay duration, produced independent measures of sustained attention and working memory maintenance ability.

Discrimination training proceeded over several phases. The operant chamber was illuminated for the entire session via the houselight. A total of 68 trials were presented per session (vITIs; μ=45s). Training details are available in supplementary materials (S1.2).

Tissue collection. Rats were decapitated without anaesthesia. The brains were rapidly removed and left in cold saline (4 °C) for at least 45 s before PFC was dissected freshly on ice. The tissue was weighed, homogenized in ice-cold 10% perchloric acid (~ 50 mg wet weight per millilitre) and centrifuged at 10,000 rpm for 10 min at 4 °C to precipitate protein. The supernatants (the perchloric acid extracts) were frozen immediately and stored at − 80 °C until the assays.

Neurochemical procedures. Determination of L-arginine, L-citrulline, L-ornithine, glutamine, GABA, glutamate, spermidine and spermine were carried out by the high performance liquid chromatography (HPLC) method, while agmatine and putrescine were measured by a highly sensitive liquid chromatography mass spectrometric (LC/MS) method (Liu et al., 2016; Zhang et al., 2018). High purity external and internal standards were used (Sigma, Sydney, Australia). All other chemicals were of analytical grade. Assay details available in supplementary materials (S1.3). The samples from all four groups were assayed at the same time in a counterbalanced manner. The assays were performed in duplicate, and values were expressed as μg/g wet tissue.

DATA ANALYSIS

Statistical analyses were completed using IBM SPSS (v23) and R (v4.0.5).

Bisection. To assess temporal bisection performance between groups, we analysed a) the proportion of responses made on the "long" lever in response to both 2 and 8 second anchor cues, and b) choice response latency, both as a function of each choice training phase. Note that for analysis of training and training latency data, data from the 100% no correction training phase was split into the first and last halves (subsequently referred to as the first and second epochs). In addition, we analysed the proportion of responses on the "long" lever over all intermediate sample and anchor cue durations (average of final five sessions; two MIA rats were excluded from intermediate analysis due to poor anchor cue retention [<80% correct]). Head entry (HE) latency, and choice response (CR) latency as a function of training and intermediate cue durations were also analysed (latency data from 32/40 rats was available due to a programming error). Data were analysed using repeated measures (rm) ANOVAs with appropriate factors, as well as independent t-tests.

***Curve fitting*.** In order to further characterize differences in our timing data, we applied the Pseudo-Logistic Model (PLM: Killeen et al., 1997), which captures PSE and WF (scalar variability), in conjunction with multiple sources of variability (non-scalar and constant), and has been found to provide a superior fit for supra-second temporal bisection data relative to traditional generalized logistic functions (Lusk et al., 2020). The PLM is given as

$$P(R_L) = \left[1 + \exp\left(\frac{T_{\frac{1}{2}} - t}{\frac{\sqrt{3}}{\pi}\sigma_t}\right)\right]^{-1}$$

whereby $T_{\frac{1}{2}}$ reflects the PSE/bisection point, and σ_t is the coefficient of variation resulting from sources of scalar (γ), non-scalar (ρ), and constant (c) variability (Killeen & Weiss, 1987), given as

$$\sigma_t = \sqrt{(\gamma t)^2 + \rho t + c^2}$$

The PLM was fit to data from the final five intermediate sessions for each rat. Group differences were investigated using independent t-tests.

***Cognition*.** Proportion of correct responses, as well as HE and CR latency at each level of the sustained attention (SA) and working memory (WM) task was calculated [average of final three sessions] and compared using rmANOVA with appropriate factors. The gradient of a linear regression respective to each cognitive experiment was calculated for each rat. Gradient values were averaged to compare between groups (t-test). Individual gradient values, as metrics of overall SA and WM ability were used in correlations (Pearson's). Note a larger gradient value indicates a steeper slope, suggesting poorer overall ability. For the PSE x SA comparison, one rat was excluded as a multivariate outlier.

Arginine. Two-way ANOVA were used to compare assayed metabolites between groups. Pearson's correlations were used to assess the relationship of metabolites with behavioural measures. Correlations were performed across all rats, and then respective to treatment group or GD group as appropriate.

RESULTS

Gestation day of treatment was not identified as contributing factor in timing, SA, or WM experiments (ps>0.1), therefore behavioural data was collapsed across gestation day within treatment groups for subsequent analysis.

Temporal bisection training. Primary analyses of training data (n=40 [20MIA]; rmANOVA, factors: training phase, cue duration, and group) did not find differences in task acquisition between MIA and control rats, indicating there was no fundamental learning deficit (fig1). We found that animals were able to discriminate between the 2 and 8s anchor cues (main effect duration; $F(1,114) = 990.17$, $p = < 0.001$), becoming more adept at discriminating as training progressed (duration x training phase; $F(3,114) = 61.73$, $p < 0.001$). Results suggested that groups were responding differently to the progressive training phases (group x training phase; $F(3,114) = 3.16$, $p = 0.03$), therefore we compared group performance across the four training stages independently for 2 and 8s durations in order to locate the difference. No differences were found in the 2s analysis (group, group x training phase; ps>0.7), however results indicated that groups were responding differently to training on the 8s cue (group x training phase; $F(3,114) = 2.70$, $p = 0.049$). As seen in fig. 1.A, this difference occurred in the 50% response phase, which was the first exposure rats had to choice trials. As such, any group differences in this phase likely reflect behavioural adaption to changes in the operant schedule rather than any fundamental difference in acquisition ability.

The absence of group differences in task acquisition is supported by analysis of CR latencies, (rmANOVA; factors: training phase, group; n=32 [16MIA]) which found that discrimination speed increased as training progressed (main effect of training phase; $F(3,90) = 116.12$, $p < 0.001$), in the absence of any group differences (group, group x training phase; Fs >2, ps > 0.6; fig1.B). In all, the training data indicates (1) that rats were able to discriminate between the anchor cues, (2) that acquisition over the different training phases did not differ between groups, and (3) that performance was equivalent between groups prior to the intermediate testing phase.

Temporal bisection testing. Analysis of responses to the intermediate durations (rmANOVA, factors: cue duration, group; n=38 [18MIA]) supported the hypothesis that MIA impairs perception of time (fig2.A). Animals were found to be sensitive to the different durations (main effect of duration; $F(6,216) = 521.47$, $p = <0.001$), however MIA rats responded differently to controls (main effect of group; $F(1,36) = 4.70$, $p = 0.04$) systematically over the intermediate durations (cue duration x group; $F(6,216) = 1.27$, $p = 0.27$). Groups were not found to differ in either CR latency (duration cue x group $F(6,180) = 0.723$, $p = 0.632$), or HE latency ($t(30) = 1.145$, $p = 0.261$) during intermediate testing, suggesting decision making and motivation factors were equivalent.

PLM. Between-group comparisons (t-tests; n=38 [18MIA]) of the parameters derived from application of the PLM to the intermediate data supported the hypothesis that MIA rats experience abnormal perception of time (fig2.B). We found moderate evidence of an increased PSE in MIA rats relative to controls ($t(36) = 1.88$, $p = 0.069$) , in the absence of changes in WF ($t(36)=1.19$, $p = 0.24$), non-scalar variability ($t(36) = 1.26$, $p = 0.22$) , and constant variability ($t(36) = 1.2$, $p = 0.24$).

Sustained attention. Based on the analysis of the sustained attention task (rmANOVA; factors: duration, group, n=40 [20MIA]), and comparison of interpolated gradients (t-test), our data do not support the hypothesis that MIA impairs sustained attention function (fig3.A). The decline in correct responses over the levels of the task indicates that decreasing cue duration was increasing the demand placed upon sustained attention capabilities (main effect of duration, $F(4,152)=162.71$, $p = <0.001$). This response however did not differ between groups (main effect of group: $F(1,38)=0.22$, $p = 0.88$; group x duration $F(4,152) = 0.99$, $p = 0.41$). Additionally, we did not find any differences in the rate of attentional decline (gradient) between groups ($t(38)=1.56$, $p = 0.13$). HE latency decreased as a function of duration (main effect of duration: $F(4,152) = 71.33$, $p<0.001$) similarly for both groups (duration x group: $F(4,152) = 0.48$, $p = 0.75$), indicating that motivation increased as the task became more difficult. CR latency increased as a function of duration (main effect of duration: $F(4,152) = 6.16$, $p<0.001$), however MIA rats took longer to respond than controls (main effect of group: $F(1,38) = 5.09$, $p = 0.03$; fig3.C).

Working memory maintenance. Results from the analysis of the WM maintenance task (rmANOVA, Factors: delay, group: n=40 [20MIA]) and the WM maintenance gradients (t-test) support the hypothesis that MIA impacts WM function (fig3.B). Discrimination ability prior to commencement of the WM task was equivalent between groups ($t(38) = 0.47$, $p = 0.64$). The decline in discrimination rates indicates that that increasing response delay increased the demand placed on WM maintenance (main effect of delay: $F(3,114) = 111.97$, $p<0.001$), however we found moderate evidence to indicate that discrimination was poorer in MIA than control rats (main effect of group: $F(1,38) = 3.408$, $p = 0.073$). Decline rates (gradients) of WM maintenance were not found to differ between groups ($t(38) = 1.392$, $p = 0.172$). Groups did not exhibit differences in motivation (HE latency: main effect of group: $F(1,38) = 0.54$, $p = 0.47$), which increased as a function of task difficulty (main effect of duration: $F(3,114) = 25.473$, $p<0.001$). CR latency did not differ as a function of delay (main effect of delay: $F(3,114) = 0.814$, $p = 0.48$), however MIA rats took longer to respond than controls (main effect of group: $F(1,38) = 4.545$, $p = 0.04$, fig3.D).

Cognition x timing. The outcome Pearson's correlations supported the hypothesis that attention and temporal perception are functionally related in both healthy and impaired instances (PSE x sustained attention gradient; n = 37 [17 MIA] fig3.E). We found a positive general relationship between temporal perception and attention ($r(35)=0.4$, $p = 0.01$), which persisted when data were then analysed independently respective to MIA ($r(16)=0.46$, $p = 0.05$) and control ($r(17)=0.52$, $p = 0.02$) treatment groups.

Analysis of the relationship between PSE and WM gradients (Pearson's correlations, n=38) did not support the hypothesis that working memory maintenance and temporal perception are related (PSE x WM maintenance gradient; n=38 [18 MIA] fig3.F). PSE and WM gradients were not generally related

(r(36)=-0.1, p = 0.56), nor when considered respective to treatment group (MIA: r(17)=0.1, p = 0.69; CON: r(17)=-0.22, p=0.35).

Arginine. Comparisons of HPLC and LC/MS measures (two-way ANOVA, Factors: GD, treatment group: n=40 [10 MIA10, 10 MIA18]) provided preliminary evidence supporting the hypothesis that MIA induces changes in arginine metabolism in PFC (results summarised in table 1). Analyses indicated that MIA animals had elevated L-citrulline and putrescine levels in PFC relative to controls (main effect of treatment, ps<0.06;). In addition, we found evidence of a time specific effect of treatment on glutamate in PFC; glutamate levels were reduced in GD10 MIA rats, and elevated in GD18 MIA rats relative to GD respective controls (GD x treatment, p = 0.06). Finally, we found evidence of main effects of GD; within PFC we identified higher L-ornithine (p = 0.07) and lower agmatine (p = 0.002) levels in GD10 animals relative to GD18s.

Arginine x behaviour. Pearson's correlations between assayed metabolites and behavioural measures (PSE n= 38, SA gradient and WM gradient n= 40) provided preliminary evidence supporting the hypothesis that arginine metabolism is linked to disease phenotypes (see table S1 for full results). L-citrulline and putrescine were found to negatively correlate with WM maintenance generally (ps<0.02). MIA rats appeared to account for this effect (L-citrulline x WM, r(18)=-0.44, p = 0.05; putrescine x WM, r(18)=-0.52, p = 0.02) in the absence of any relationship between these measures for control animals (ps>0.2; fig4.A&B).

The relationship between agmatine, L-ornithine, glutamate and behavioural measures was analysed respective to GD treatment group (see table S2 for full results). We found evidence of a positive correlation between L-ornithine and PSE for both GD10 MIA (r(9)=0.68, p = 0.03) and control rats (r(8)=0.59, p = 0.09), as well as evidence of a negative correlation between L-ornithine and PSE for GD18 control animals (r(9)=-0.6, p = 0.06) in the absence of a relationship for GD18 MIA rats (r(8)=-0.54, p = 0.13, see fig4.C&D).

In addition, we identified a positive relationship between L-ornithine and SA capacity for GD10 MIA rats (r(9)=0.69, p = 0.03), which was not apparent in GD10 controls (r(9)= 0.27, p = 0.44). L-ornithine and SA capacity was negatively correlated in GD18 control rats (r(9)=-0.65, p = 0.03), however no relationship was found in GD18 MIA rats (r(9)=-0.06, p = 0.86, see fig4.E&F)). Finally, we found a significant positive relationship between glutamate and SA capacity exclusively in GD18 MIA animals (r(9)=0.69, p = 0.03).

DISCUSSION

Summary of results

Here we profiled the impact of MIA on temporal perception, cognition, and the arginine metabolic profile in PFC, as well as the interrelationship between these measures. First, we identified that MIA rats experienced a subjective quickening of temporal perception relative to sustained attention capacity; rats with poorer attention experienced greater temporal underestimation. Second, MIA exposure induced deficits in working memory maintenance. Third, MIA produced both general and time specific impacts on arginine metabolism in PFC, of which related to sustained attention and working memory maintenance functions. In addition, we found involvement of L-ornithine in timing-attention functions irrespective of treatment. Finally, inciting MIA either early or late in gestation was found to have a limited and subtle impact on outcomes measured. These findings serve to further characterise the complex psychopathophysiology specific to prenatal immunological damage as a precursor for psychiatric disease.

Sustained attention capacity mediates abnormal timing resulting from MIA

The ability of MIA to incite temporal perception abnormalities is consistent with our previous work with GD15 rats (Ashley R. Deane et al., 2017). Notably the effect direction in the present instance differed as rats underestimated temporal passages, corresponding to a subjective quickening of time. The basis for this difference is unclear, however the direction and magnitude of the shift in the present data is analogous to bisection results from rats administered DA agonists (Meck, 1986), and clinical investigations (Thoenes & Oberfeld, 2017). This indicates that the model is accurately recapitulating disease traits observed in schizophrenia.

Our isolation of a relationship between SA capacity and timing accuracy supports previous research implicating attention mechanisms in regulating temporal pace (de Montalembert et al., 2016). We found that irrespective of health status, temporal perception shortened as a function of reduced SA capacity, suggesting either a functional interrelationship between timing and attention, a common instantiating mechanism, or both. Consistent with previous findings from our lab (Bates et al., 2018), MIA rats did not exhibit a basal impairment in attention, yet their underestimation of temporal events corresponded with attentional capacity, supporting the idea that severity of cognitive impairment predicts the magnitude of temporal impairment in schizophrenia (Lee et al., 2009; Papageorgiou et al., 2013). Bisection findings support the absence of a basal impairment in attention, as MIA was not found to increase perceptual variability (flattening of the psychophysical function). To our knowledge, this is the first direct evidence of a timing-attention relationship in rodents.

The absence of a relationship between timing and WM in the present study was unusual, particularly given the WM maintenance impairment in MIA rats, as prospective timing accuracy is regarded to

rely heavily on WM ability (Wiener et al., 2010). One possible account is that the short cue durations (2-8 s) in combination with immediate lever extension in the bisection task exerted minimal demand upon WM capacity, and as such our bisection findings do not index this function, accounting for the absent timing-WM relationship. If WM recruitment in timing does increase proportionate to the complexity of the stimulus, testing longer durations in the bisection task may better isolate this effect. The presence of a WM deficit is consistent with previous MIA findings (Murray et al., 2017).

While this research did not directly investigate the neurobiology of timing deficits in MIA rats, our set of findings provide potential clues to this end. Given previous evidence of increased DA in both MIA rodents and patients with schizophrenia (Howes et al., 2012; Winter et al., 2009; Zuckerman et al., 2003), as well as strong evidence of increased DA activity mediating temporal underestimation (Meck, 1986; Soares et al., 2016), interpreting present findings within this context implicates increased DAergic activity as a candidate mechanism informing temporal underestimation in MIA rats. Critically, however, DAergic activity is unlikely to directly explain our effect; basal dysregulation would not produce the systematic rightwards shift in the psychometric profile of MIA rats, as this deficit would impact all timed durations, not only intermediate cues. Moreover, it is yet unclear whether DA neural activity has a causal role in timing, or is simply reflecting temporal function (Soares et al., 2016). On this basis, elevated DA activity, rather than being a direct instantiating mechanism, may contribute to impaired timing via direct involvement in, or indirect dysregulation of, overlapping functions. Notably, states associated with increased DA activity, including learning, motivation, and cognitive engagement, have been linked to healthy perceptual underestimation (Fried et al., 2001; Gable & Poole, 2012). Owing to the paucity of research examining the neural correlates of temporal perception deficits in schizophrenia, future examination of this area is warranted.

Arginine metabolism is implicated in timing and cognitive outcomes following exposure to MIA

We found evidence indicating that within PFC, exposure to MIA produced generalised increases in L-citrulline and decreases in putrescine, as well as time specific effects on glutamate. Relative to controls, glutamate in PFC was reduced following MIA at GD10 and increased following MIA at GD18. These findings are consistent with previous work highlighting the impact of MIA on arginine metabolism; GD15 rats were reported to have elevated L-arginine, L-ornithine and putrescine and decreased agmatine in the hippocampus and PFC (Jing et al., 2013). This, however, represents the first suggestion of a time specific effect of MIA on arginine metabolism. We also found that time of injection (GD) differently impacted PFC L-ornithine and agmatine, irrespective of treatment. The basis of this difference is unclear, although one possible account is that anaesthesia, or maternal stress resulting from the injection procedure, may differentially impact offspring depending on when this occurs in gestation.

Although altered brain arginine metabolism is an established disease feature of schizophrenia (Liu et al., 2016), there is a paucity of research investigating the involvement of arginine metabolism in functional outcomes. Previously, PFC L-arginine has been linked with healthy rodent prepulse inhibition (PPI), a marker of sensorimotor gating; notably this relationship was absent for MIA rats (Jing et al., 2013). Present results provide preliminary evidence suggesting that changes in arginine metabolism are linked to timing and cognitive outcomes following MIA exposure. Increases in both L-citrulline and putrescine corresponded with poorer WM maintenance in MIA rats, in the absence of any relationship between these factors for control animals.

Our findings indicated that involvement of PFC L-ornithine in timing and attention is dependent on both treatment and time of injection during gestation. We found evidence that L-ornithine was related to both temporal accuracy and SA capacity in GD10 MIA and GD18 controls, and exclusively related to timing in GD10 controls, however correlation trends were positive for GD10 animals, and negative for GD18s. No relationship between these measures was found for GD18 MIA animals. The relationship of L-ornithine with timing and attention, regardless of directionality, raises the possibility of L-ornithine being directly related to the biological instantiation of time, or being regulated by a timing-related mechanism. L-ornithine has previously been implicated in regulating circadian timing of both rodents and humans (Fukuda et al., 2016, 2018), however this is the first evidence relating L-ornithine to temporal perception, and as such future investigations are warranted. Finally, we found that SA capacity reduced as a function of increasing PFC glutamate levels in GD18 MIA rats. In all, these preliminary results, in conjunction with evidence of the critical involvement of PFC in timing and cognition (Gómez et al., 2014), support the idea that changes in PFC are central to the neuropathophysiology of schizophrenia.

Time-specific MIA in rats does not produce discrete symptom profiles

The paucity of time-specific MIA effects in this study is informative regarding the gestational window of vulnerability hypothesis in MIA rats. While we did find that MIA impacted glutamate in a time-specific manner, which related to SA functioning following GD18 MIA, the majority of our findings were not influenced by time of exposure to MIA. This outcome conflicts with the time-specific disease characteristics observed in the MIA mouse model (Urs Meyer et al., 2006, 2008), although it is congruent with trends emerging from the early/late MIA rat research, which suggest that time-specific exposure to MIA produces a narrowed and attenuated disease profile rather than time-specific pathology (da Silveira et al., 2017; Deane et al., 2021; Hao et al., 2019; Rahman et al., 2020). While more research in this area is needed, preliminary interpretation is that the magnitude of vulnerability to MIA at analogous time points differs between species, and as such care should be taken when generalising murine findings on this topic. These species-specific outcomes will likely have implications for future use of the model in etiopathological and pharmacological investigations.

Conclusion statement

In all, these findings provide clues about the biological instantiation of pathological timing following exposure to an inflammatory risk factor for schizophrenia. Findings build on previous work implicating attentional mechanisms in timing through presenting the first direct isolation of this functional relationship in both healthy and pathological instances. These outcomes commend the MIA rat model as a tool to elucidate the biological bases of temporal perception impairment in future. In addition, we report preliminary evidence of a relationship between arginine metabolism and cognition following exposure to MIA, as well as evidence that L-ornithine may be involved in timing-attention functions generally. As L-arginine is metabolically versatile, altered metabolism likely has broad functional implications and warrants increased attention within schizophrenia research.

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FIGURES

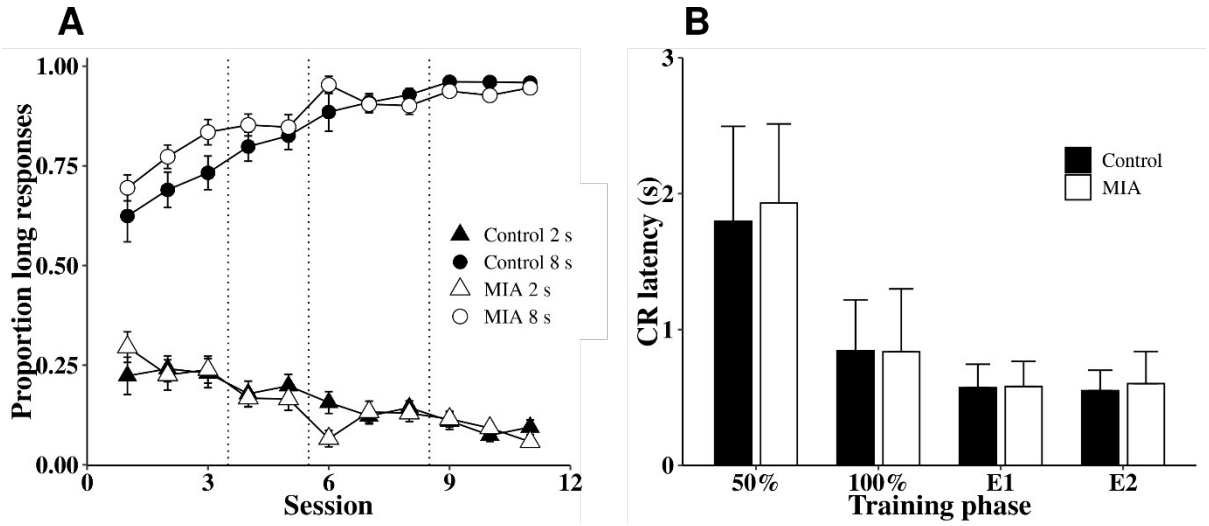

Figure 1. Bisection training. **(A)** Mean proportion of responses on the lever corresponding to the "long" cue during both 2 and 8 second anchor cue trials. **(B)** Choice response (CR) latency (averaged over anchor cue durations). Data are shown across training phases (50% choice, 100% choice with correction, 100% choice without correction (first and second epochs [E1/E2]). Error bars indicate SEM.

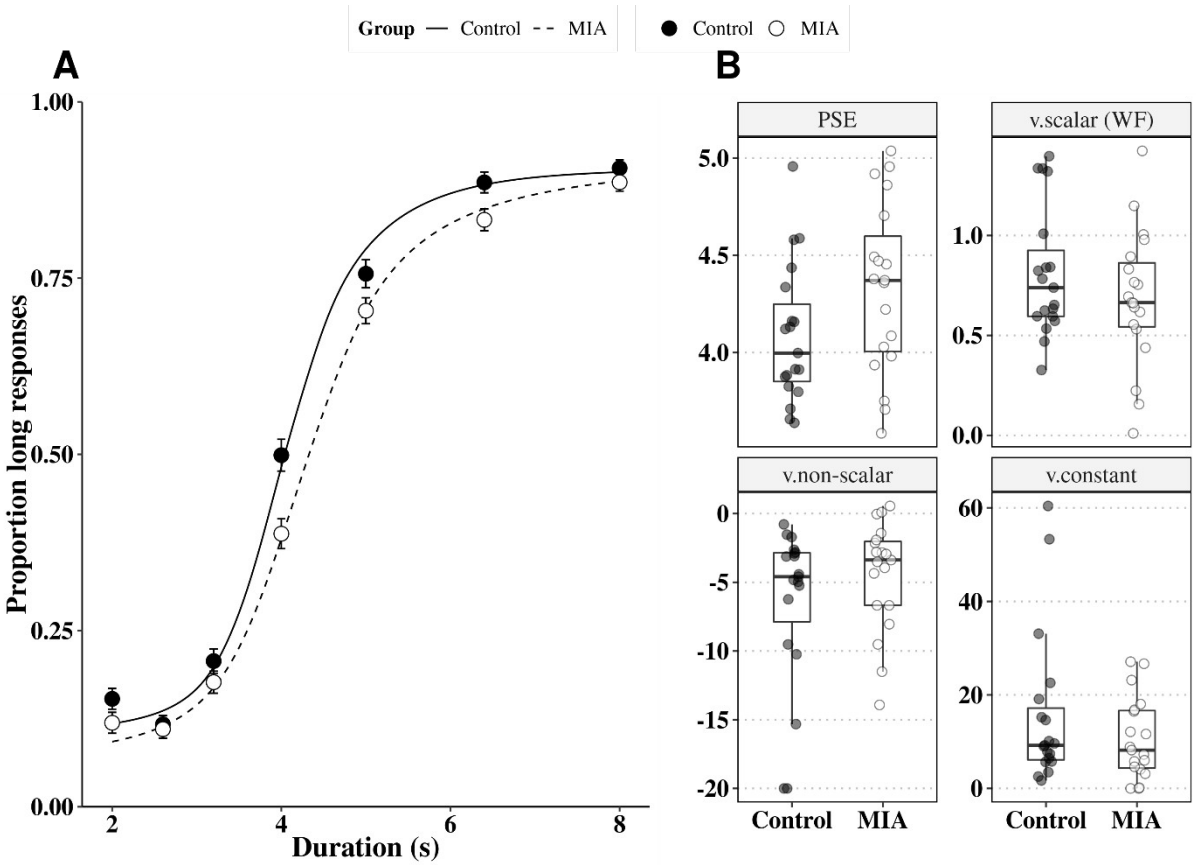

Figure 2. Intermediate bisection testing. **(A)** Mean proportion of responses on the lever corresponding to the "long" anchor cue as a function of sample duration for control and MIA rats. The lines through the data are the best fitting form of PLM to the average proportion-long response data across rats from each group (parameter estimates are based on fits of the model to individual rats). Error bars reflect SEM. **(B)** Mean best fitting parameters derived from fitting the PLM to the proportion long data from control and MIA rats; point of subjective equality (PSE), scalar, non-scalar and constant variability.

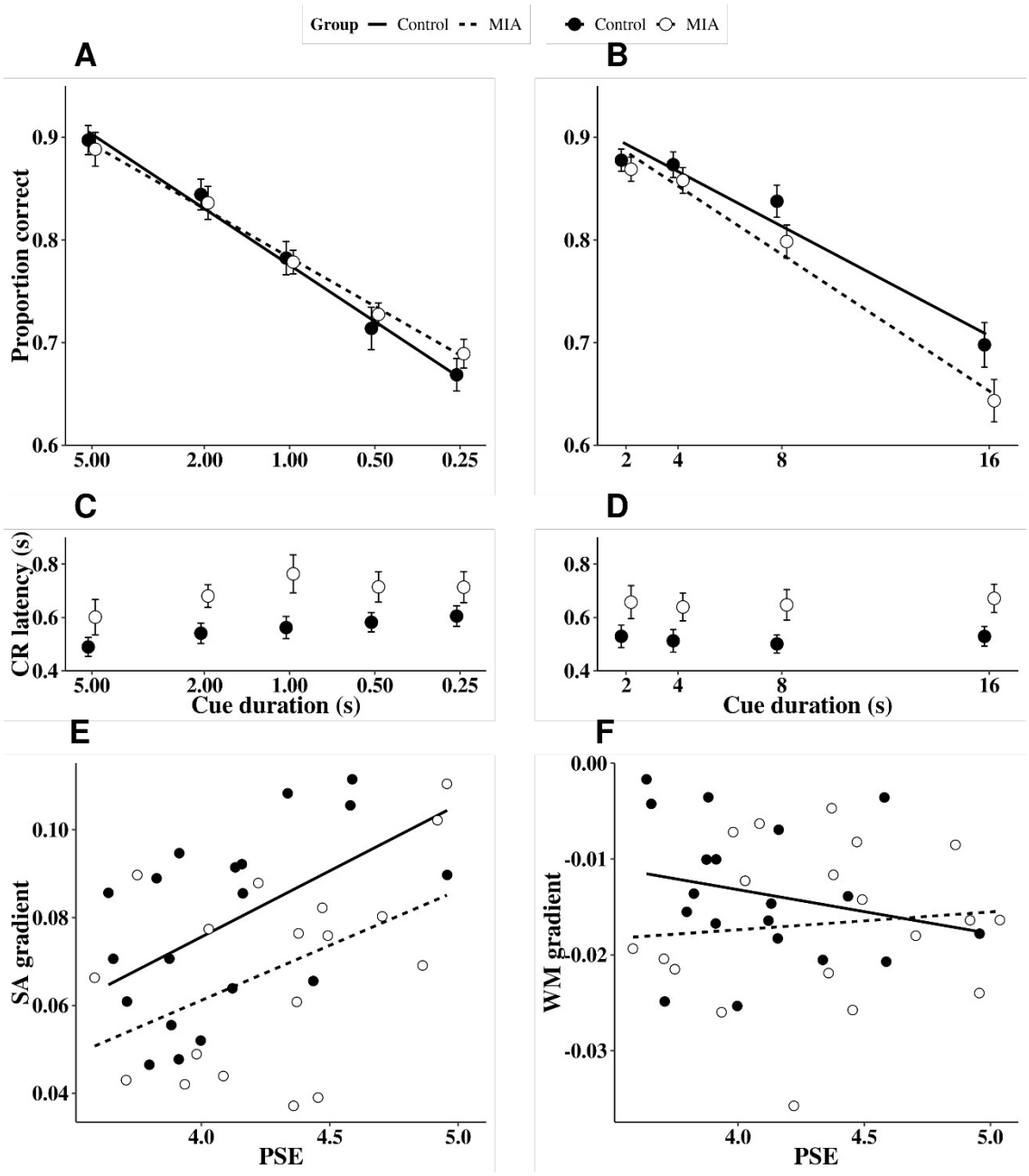

Figure 3. Cognitive capacity and related involvement in timing. Sustained attention (SA): **(A)** average proportion of correct responses, and **(C)** choice response (CR) latency, as a function of declining cue duration for treatment groups. Working memory (WM): **(B)** average proportion of correct responses, and **(D)** choice response latency, as a function of increasing lever extension delay for treatment groups. For both experiments, regression lines indicate the average rate of decline for each group (note a log10 transform has been applied to (A)). Panels **(E)** and **(F)** show the relationship between cognitive gradients and PSE for individuals respective to treatment group. Error bars indicate SEM.

Table 1. Summary statistics and two-way ANOVA results comparing L-arginine metabolites between GD10 and GD18 MIA and control rats

	ARG		ORN		Glutamine		CIT		GLU		GABA		AGM		PUT		SPD		SPM		SPD/SPM	
	u	**sd**	**u**	**sd**	**u**	**sd**	**u**	**sd**	**u**	**sd**	**u**	**sd**	**u**	**sd**	**u**	**sd**	**u**	**sd**	**u**	**sd**	**u**	**sd**
CON10	47.79	4.90	54.98	13.08	1597.24	173.37	414.41	33.29	5126.97	381.99	311.63	35.54	0.03	0.00	0.25	0.10	26.38	3.15	35.86	7.36	1.05	0.15
MIA10	48.29	4.67	54.60	13.42	1640.28	83.24	432.08	39.03	5006.28	333.00	319.03	29.89	0.03	0.01	0.27	0.13	28.13	6.67	35.05	9.58	1.15	0.17
CON18	46.29	4.23	47.56	9.28	1574.99	183.32	399.50	38.73	4931.84	350.50	299.44	25.47	0.04	0.01	0.21	0.04	24.92	3.25	34.19	10.44	1.08	0.25
MIA18	46.64	4.54	48.57	9.90	1605.91	179.01	438.69	38.52	5220.68	243.12	306.84	21.56	0.04	0.01	0.30	0.08	26.98	3.21	36.43	8.59	1.06	0.16
df = (3,36)	**F**	**sig.**	**F**	**sig.**	**F**	**sig.**	**F**	**sig.**	**F**	**sig.**	**F**	**sig.**	**F**	**sig.**	**F**	**sig.**	**F**	**sig.**	**F**	**sig.**	**F**	**sig.**
Treatment	0.09	0.77	0.01	0.93	0.53	0.47	5.76	**0.02**	0.64	0.47	0.67	0.42	0.01	0.95	3.97	**0.05**	1.93	0.17	0.06	0.81	0.49	0.49
GD	1.18	0.29	3.38	**0.07**	0.31	0.58	0.12	0.73	0.01	0.93	1.82	0.19	10.79	**0.002**	0.08	0.78	0.90	0.35	0.00	0.96	0.18	0.68
Treatment*GD	0.00	0.96	0.04	0.85	0.01	0.91	0.82	0.37	3.82	**0.06**	0.00	1.00	0.18	0.67	1.08	0.31	0.01	0.91	0.28	0.60	0.93	0.34

Means reflect assayed metabolite levels (ug/g wet tissue). ARG = L-arginine; ORN = L-ornithine; CIT = L-citrulline; GLU = glutamate; GABA = Gamma aminobutyric acid; PUT = putrescine; SPD = spermidine; SPM = spermine; AGM = agmatine

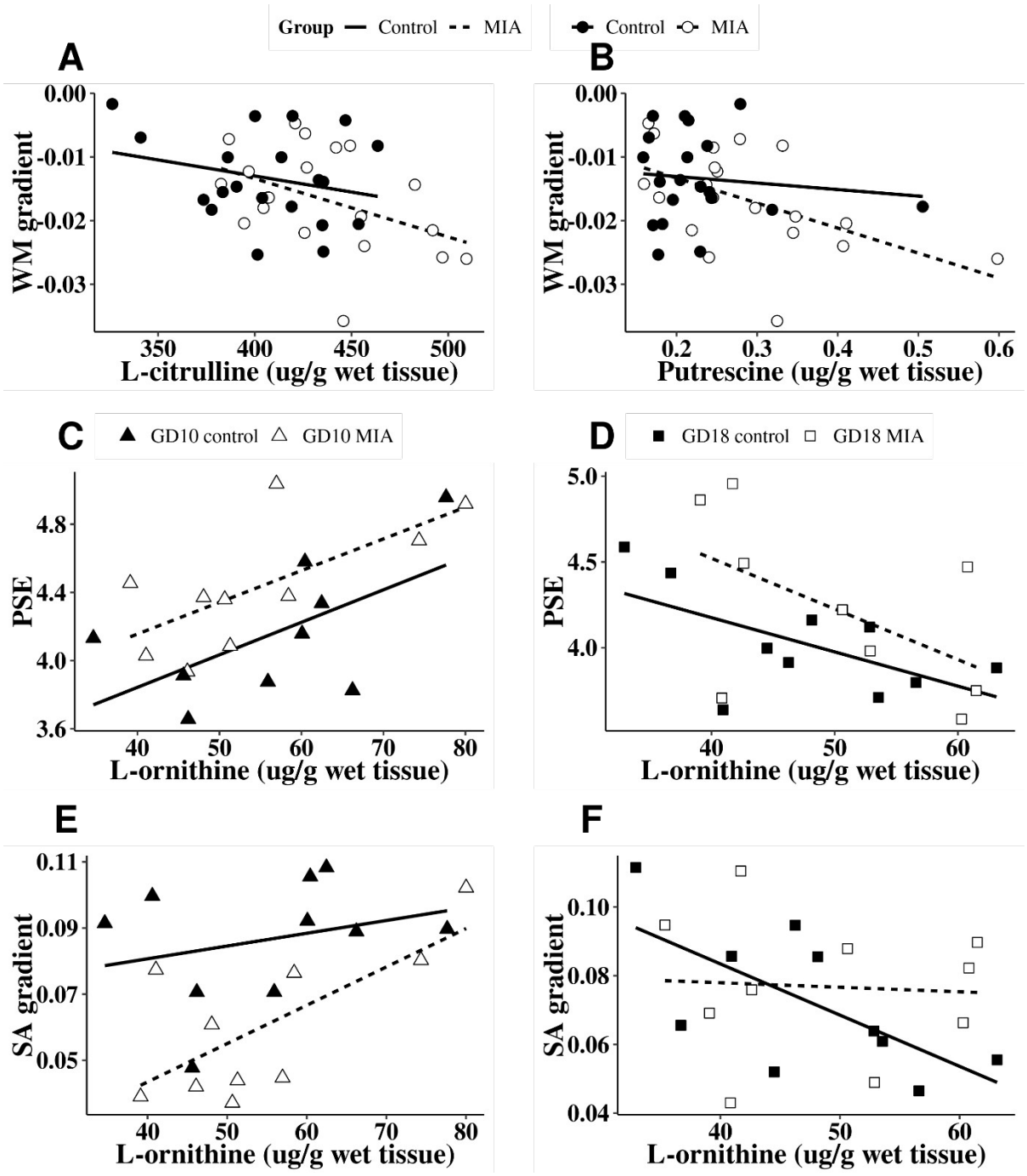

Figure 4. Cognitive function and arginine metabolism. Working memory (WM): relationship with **(A)** L-citrulline and **(B)** putrescine, respective to treatment group. PSE: relationship with L-ornithine for **(C)** GD10, and **(D)** GD18 rats respective to treatment groups. Sustained attention (SA): relationship with L-ornithine for **(E)** GD10, and **(F)** GD18 rats respective to treatment groups. Trend lines reflect linear regression of data points respective to group.