DR. PATRICIA V. AGOSTINO (Orcid ID : 0000-0003-4386-2228)

Article type : Special Issue Article

Manuscript # EJN-2017-03-24446 Rev.2

Deficits in temporal processing in mice prenatally exposed to Valproic Acid

Julieta Acosta[1], Marcos A. Campolongo[2], Christian Höcht[3], Amaicha M. Depino[2], Diego A. Golombek[1] and Patricia V. Agostino[1*]

[1]Laboratorio de Cronobiología, Universidad Nacional de Quilmes/CONICET. [2]Instituto de Fisiología, Biología Molecular y Neurociencias, CONICET-UBA. [3]Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, UBA. Buenos Aires, Argentina.

Running title: Interval timing in a mouse model of autism

Keywords: autism spectrum disorder, cortico-striatal circuits, dopamine, interval timing, valproic acid.

*Corresponding author:

Departamento de Ciencia y Tecnología

Universidad Nacional de Quilmes

Roque Sáenz Peña 352

Bernal (B1876BXD), Buenos Aires

ARGENTINA

Tel: +54-11-4365-7100 ext. 5626

Fax: +54-11-4365-7132

E-mail: pagostino@unq.edu.ar

Abstract

Temporal processing in the seconds-to-minutes range, known as interval timing, is a crucial cognitive function that requires activation of cortico-striatal circuits via dopaminergic-glutamatergic pathways. In humans, both children and adults with autism spectrum disorders (ASD) present alterations in their estimation of time intervals. At present, there are no records of interval timing studies in animal models of ASD. Hence, the objective of the present work was to evaluate interval timing in a mouse model of prenatal exposure to valproic acid (VPA) – a treatment used to induce human-like autistic features in rodent models. Animals were assessed for their ability to acquire timing responses in 15-s and 45-s peak-interval (PI) procedures. Our results indicate that both female and male mice prenatally exposed to VPA present decreased timing accuracy and precision compared to control groups, as well as deviations from the scalar property. Moreover, the observed timing deficits in male VPA mice were reversed after early social enrichment. Furthermore, catecholamine determination by HPLC-ED indicated significant differences in striatal dopaminergic, but not serotonergic, content in female and male VPA mice, consistent with previously identified alterations in dopamine metabolism in ASD. These deficits in temporal processing in a mouse model of autism complement previous results in humans, and provide a useful tool for further behavioral and pharmacological studies.

Introduction

The ability to sense the passage of time is a fundamental component of cognition. Duration discrimination within the seconds-to-minutes range, known as interval timing, is critical for fundamental behaviors such as foraging, decision-making and learning (Meck, 2003; Buhusi & Meck, 2005). Although the exact mechanisms of temporal processing in the brain have not been completely elucidated, it has been established that interval timing involves the interaction of the basal ganglia and the prefrontal cortex via dopaminergic-glutamatergic pathways (Matell *et al*., 2003; Lustig *et al*., 2005; Coull *et al*., 2011). Manipulations of these dopaminergic systems are able to modify interval timing by altering the speed and other properties of the internal clock (Jones & Jahanshahi, 2011; Balci, 2014; Agostino & Cheng, 2016; Soares *et al*., 2016). In this sense, deficits in timing functions have been reported in disorders associated with pathological dopaminergic function, including Parkinson's disease, Huntington's disease, and multiple system atrophy (Malapani *et al*., 1998; Beste *et al*., 2007; Högl *et al*., 2014; Agostino *et al*., 2017).

Autism spectrum disorders (ASD) are characterized by impairments in social interactions, communication deficits, and restricted repetitive and stereotyped interests and behaviors. Besides these core-behavioral aspects that define the pathology, individuals with ASD present a variety of secondary symptoms such as attentional deficits, anxiety, sleep problems and motor dysfunction (American Psychiatric Association, 2013). Among these, the ability of perceiving the passage of time is also affected in ASD (Boucher, 2001; Allman *et al*., 2011). Specifically, short time estimation is altered in both children (Szelag *et al*., 2004: Allman & DeLeon, 2009) and adults (Martin *et al*., 2010) with ASD. Furthermore, neuroimaging studies in autistic patients have shown abnormalities in the structure and function of the prefrontal cortex, basal ganglia and cerebellum (Allman & Meck, 2012). In addition, disruptions in normal dopaminergic and serotonergic transport systems (Nakamura *et al*., 2010) have also been reported in ASD. All these findings indicate that the neuroanatomical and neurochemical basis underlying interval timing may be altered in individuals with ASD.

Despite the intense research focused in autism in the recent years, motivated by the increasing number of children diagnosed with ASD – about 1 in 28 (Kim *et al*., 2011; Zablotsky *et al*., 2014) – the etiology of autism remains still unknown. Several lines of evidence highlight the role of environmental factors interacting with predisposing genetic conditions as the predominant cause of ASD (Mandy & Lai, 2016). In particular, *in utero* exposure to teratogenic agents, such as thalidomide or valproic acid (VPA), have been profoundly implicated in increasing the risk of undergoing autism (Stromland *et al*., 1994; Moore *et al*., 2000; Bromley *et al*., 2008). Thereby, pharmacological treatment using drugs that affect normal development (i.e. prenatal administration) has been used to generate animal models of autism (Schneider & Przewłocki, 2005; Wagner *et al*., 2006; Dufour-Rainfray *et al*., 2011). These models exhibit reduced social interaction, as seen in rats (Schneider & Przewłocki, 2005; Kim *et al*., 2011) and mice (Wagner *et al*., 2006; Lucchina & Depino, 2014), in a similar manner to that observed in children prenatally exposed to VPA. Importantly, the murine VPA model of autism fits the criteria of construct and face validity (Belzung *et al*., 2005; Patterson, 2011; Lucchina & Depino, 2014).

At present, the impact of ASD in temporal perception in animal models is unknown. Consequently, a mouse model of autism generated by prenatal exposure to VPA at gestational day 12.5, was evaluated for its ability to time short intervals using two target durations (15-s and 45-s) in the peak-interval (PI) procedure (Cheng & Meck, 2007; Agostino *et al*., 2013). This model has the advantage of presenting a male/female difference in the effect of prenatal VPA exposure, coincident with the observed bias in ASD (higher risk in boys than girls, with an incidence ratio of 4:1), as only male VPA mice present the reduction in sociability (Kataoka *et al*., 2013). A similar phenotype was also described in prenatally VPA-exposed rats (Schneider *et al*., 2008; Kim *et al*., 2013). Moreover, we have found that VPA-exposed male mice that are weaned with control mice do not show the reduction in sociability observed in VPA-exposed mice reared with other VPA mice (reversion of the low sociability phenotype by early social stimulation), as was previously shown in another model of ASD (Yang *et al*., 2011).

Consequently, interval timing accuracy, precision, scalar property and neurochemical substrates were evaluated in female and male VPA-exposed mice.

Materials and Methods

Animals

Male and female CF1 mice (CrlFcen:CF1 outbred strain) were purchased from commercial suppliers (Faculty of Exact and Natural Sciences, University of Buenos Aires, Buenos Aires, Argentina). Animals were 8-10 weeks old at the beginning of the study. The murine model of prenatal exposure to VPA (referred to as "VPA model") was previously characterized and described (Lucchina & Depino, 2014; Kazlauskas *et al*., 2016). Briefly, it consists of a single subcutaneous injection of 600 mg/kg VPA to pregnant female mice at gestational day 12.5 (GD12.5). Control animals were injected with saline solution (SAL). Litters were culled to 8-10 pups and weaned at postnatal day (PD) 21 in cages containing four to six animals with the same prenatal treatment (VPA or SAL). Mice prenatally exposed to VPA show deficits in social interaction, anxiety-related behaviors, and increased levels of repetitive behaviors (Lucchina & Depino, 2014), with the exception of female VPA mice that do not show decreased levels of sociability (Kataoka *et al*., 2013). Moreover, early social stimulation can reverse the low sociability phenotype in males (Yang *et al*., 2011).

Animals were maintained in a 12:12-h light-dark cycle (LD, lights ON at 8 PM) with food and water *ad libitum* (except when noted) and room temperature set at 20 ± 2ºC. All testing was conducted during the dark period of the LD cycle, at *Zeitgeber* time (ZT) 14-18 (i.e., 2-6 hours after lights OFF). When animals had to be handled in the dark, a dim red light source (< 5 lux) was used. The present experiments were approved by the Animal Care and Use Committee of University of Quilmes (Buenos Aires, Argentina), and performed in strict accordance with NIH rules for animal care and maintenance.

Experimental groups

Female mice were divided in two groups, VPA-VPA or SAL-SAL, where the label refers to the prenatal exposure (valproic acid or saline) followed by the growing environment of the pups (valproic acid- or saline-exposed littermates). Female VPA-exposed mice were weaned with other VPA-exposed mice (VPA-VPA) and control mice were weaned with other SAL mice (SAL-SAL, n=6/group). Male mice, on the other hand, included the reversion of phenotype on social behavior by interaction of VPA-exposed animals with control peers since weaning, and were divided in four experimental groups (n=5-7/group). The labels refer to the prenatal exposure and the growing environment, respectively: mice prenatally exposed to VPA weaned with other VPA mice (VPA-VPA), mice prenatally exposed to SAL weaned with other SAL mice (controls, SAL-SAL), mice prenatally exposed to VPA weaned with SAL mice (reversion of low sociability phenotype by early social stimulation, VPA-SAL), and mice prenatally exposed to SAL weaned with VPA mice (phenotype reversion controls, SAL-VPA). The early social stimulation cages contained 2-3 VPA-exposed mice along with 2-3 SAL mice. This design allowed VPA and SAL mice to interact in the home cage from PD21 to PD60. At PD60, VPA-SAL mice exhibited higher levels of sociability than VPA-VPA mice (data not shown), indicating that this treatment can rescue at least some of the behavioral alterations observed in this model (similar to what was reported by Yang *et al*., 2011).

Apparatus

The experimental apparatus consisted of 4 matching lever boxes (Model ENV-307A, Med Associates, St. Albans, VT) housed in sound-attenuating chambers (Model ENV-021M; Med Associates, St. Albans, VT). The dimensions of each lever box were 21.59 x 17.78 x 12.70 cm. The ceiling, side walls, and door of each box were made from clear Plexiglas. The front and back walls were stainless-steel panels and the floor was made of parallel stainless-steel bars. The front wall of each box contained left and right retractable levers; a food cup was located between the levers; and a stimulus light was located directly above each lever. A pellet

dispenser delivered 20-mg grain-based food pellets (Research Diets, Inc., New Brunswick, NJ) into the food cup. The back wall of each box contained a house light (14-W, 100 mA) directed towards the ceiling. The operant chambers were controlled by the Med-PC IV software package. The fan was ON throughout the session. A PC attached to an electronic interface (MED Associates, Inc., Model DIG-700 and SG-215) was used to control the experimental equipment and record the data. The time of each lever press was recorded to an accuracy of 10-ms and placed into 1-s time bins.

Behavioral Procedures

Interval timing

Animals were trained following the peak-interval (PI) procedure as previously reported (Agostino *et al*., 2013), with slight modifications. Briefly, mice were trained in three consecutive phases: 1) operant lever press training, 2) fixed-interval training, and 3) peak-interval training. Mice were food-deprived – by controlling the amount of food they daily received – starting 7 days prior to the experiment in order to keep them at 85-90% of their free feeding weight. Animals were weighed before each session. In all segments of the experiment, sessions occurred once per day, 5 days per week.

1) Operant lever press training. All mice were given 5 daily sessions of lever-press training in which one lever – left or right, balanced among subjects – was presented during the session. Each press resulted in the delivery of a food pellet. Sessions ended after the mouse received 60 food pellets or 60 min had passed, whichever came first. All mice acquired the lever press response as indicated by the receipt of 60 food pellets.

2) 15-s and 45-s Fixed-Interval (FI) training. A dual 15-s and 45-s FI training schedule was used. The beginning of each FI trial was signaled by the lever extension and the onset of the house light. The appropriate lever was primed for reinforcement at the associated target duration (15-s or 45-s). The target duration used on each trial was randomly selected with

equal probability and no external cue was given to indicate which lever/duration was in effect. In all cases, the first response following the selected target duration resulted in the delivery of a food pellet, signal termination (i.e., lever retraction and light OFF), and the onset of a random duration inter-trial interval (ITI), range 30-110 seconds. The assignment of target durations to response levers was counter-balanced both within and across groups of mice. Animals received at least 15 sessions of FI training.

3) 15-s and 45-s Peak-Interval (PI) training. PI training was used to assess the accuracy and precision with which mice timed the target duration(s). Sessions consisted of two trial types: FI trials (as described above) and unreinforced probe trials. During probe trials the lever was extended and the light signal turned ON for 3 times the target duration. No food was available for lever pressing on these unreinforced probe trials. FI and probe trials were ordered randomly with 50% probability each. Thus, one of the two target durations (15-s or 45-s) was presented in conjunction with non-reinforced probe trials in a random sequence. No external cue was provided to indicate which, if any, lever/target duration would be selected for reinforcement on any trial. Mice were free to respond on the lever(s) at any time during the session, though only responses made to the appropriate lever following the target duration were reinforced. Animals received at least 25 sessions of PI training.

Progressive ratio (PR) schedule

The PR schedule was used to assess the amount of effort a mouse was willing to expend to get a reward, in this case a food pellet (Richardson & Roberts, 1996). Briefly, one lever was extended at the beginning of the session, and the reward was delivered only after the mouse has made a certain criterion of lever presses. The criterion was derived from the following equation:

$$P = 5 \times \exp(i \times 0.2) \ - 5$$

where ***P*** is the required number of lever presses (rounded to the nearest integer) and ***i*** refers to the reward number. This equation results in the following arithmetic series: 1, 2, 4, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145.178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, 1347, 1647, 2012, etc. Therefore, the criterion was set at one lever press for food reward the first time, two lever presses for food reward the second time, four lever presses for food reward the third time, and so forth. The session ended after 2 hours. In this test, the animal's "breaking point" was defined as the first criterion it was unable to complete successfully. Mice were evaluated in a unique 2-h session of PR schedule, the next day after completing the interval timing protocol.

Catecholamine determination

A day after completing the PR task, mice were sacrificed via cervical dislocation at ZT 18, and their brains were quickly removed under red light and kept at -80 °C. Punches of dorsal striatum from each hemisphere were taken. Tissue was homogenized in 1 ml of 0.3 M perchloric acid, centrifuged for 15 min at 3000 g at 4 °C and then frozen at 80 °C. Levels of DA, DOPAC, 5-HT and 5-HIAA were measured by high pressure liquid chromatography coupled to electrochemical detection (HPLC-ED) using a Phenomenex Luna 5 µm, C18, 250 mm × 4.60 mm column (Phenomenex, Torrance, CA, USA) and an LC-4C electrochemical detector with glassy carbon electrode (BAS). The working electrode was set at +0.65 V versus an Ag/AgCl reference electrode. The mobile phase contained 0.76 M NaH2PO4·H2O, 0.5 mM EDTA, 1.2 mM 1-octane sulfonic acid, and 5% acetonitrile; pH was adjusted to 3.0. The variation coefficient of the technique was less than 5% and the lower limit of detection of MD was 5.0 ng/ml. Intra-day and inter-day coefficient of variation was 3.2 and 13.2%, respectively. Catecholamine quantification was referred to total protein content. Proteins were measured by using the NanoDrop 1000 Spectrophotometer (Thermo Scientific).

Statistical analysis

Interval timing

Average curve analysis

Data were used to estimate the peak time, peak rate, and precision of timing from the response functions for each mouse. The number of responses (in 1-s bins) was averaged daily over trials, to obtain a mean response rate for each mouse. Daily mean response-rate functions for the interval of interest were fit using the Marquardt-Levenberg iterative algorithm (Marquardt, 1963) to find the coefficients (parameters) of a Gaussian + linear equation that gave the best fit (least squares minimization) between the equation and the data. The following model was fit to the individual daily mean response rate function for each mouse:

$$R(t) = a \times \exp(-0.5 \times \left[\frac{t - t_0}{b}\right]^2) + c \times (t - t_0) + d$$

where $\boldsymbol{t}$ is the current time, and $\boldsymbol{R(t)}$ is the mean number of responses at time $\boldsymbol{t}$. The iterative algorithm provided parameters $\boldsymbol{a}$, $\boldsymbol{b}$, $\boldsymbol{c}$, $\boldsymbol{d}$ and $\boldsymbol{t_0}$. Parameter $\boldsymbol{t_0}$ (peak time or peak position) was used as an estimate of the daily peak time of responding, $\boldsymbol{a} + \boldsymbol{d}$ (peak amplitude or peak height) was used as an estimate of the peak rate of response (being $\boldsymbol{a}$ the height of the Gaussian curve, and $\boldsymbol{d}$ the height of the linear equation), and parameter $\boldsymbol{b}$ (peak width) was used as an estimate of the precision of interval timing. These parameters were calculated for individual animals and then compared across groups by using two-tailed t-test or two-way ANOVA.

Data from the mean response rate over PI trials were also used to calculate the S1 and S2 rate indexes as previously reported (Cheng & Meck, 2007: Agostino *et al*., 2013; Bussi *et al*., 2014; Bussi *et al*., 2015). These rate indexes were calculated by taking the response rate in a specified interval (20% of the target duration) just prior to (S1) or after (S2) the expected time of reinforcement as a ratio of overall response rate within the first (S1) or second (S2) portion of the trial. For example, the S1 rate index for the PI 15-s training was defined by the response rate

occurring during the 3-s period just prior to the expected time of reinforcement (i.e., seconds 13-15) divided by the overall response rate for the first 15-s of the trial (i.e., seconds 0-15). Similarly, the S2 rate index for the PI 15-s training was defined by the response rate occurring during the 3-s period just after the time of reinforcement (i.e., seconds 15-17) divided by the overall response rate during the last 30-s of the trial (i.e., seconds 15-45). During FI training, only S1 rate index was calculated. Higher values of S1 and S2 indicate sharper FI or PI timing functions and better duration discrimination (Cheng & Meck, 2007). To analyze the S1 and S2 rate indexes across sessions, a mixed-design (two-way repeated measures) ANOVA was performed.

Single trial analysis

Analysis of responding in individual peak trials was performed as previously reported (Church *et al*., 1994; Matell & Meck, 2006; Balci *et al*., 2010; Bussi *et al*., 2015). The transition into (Start time) and out of (Stop time) the high rate was defined by the point at which the data first exceeded or fell below, respectively, 75% of the maximum response rate. Peak time was defined as the middle point between Start and Stop times: (Start + Stop) / 2. Single-trial analysis was performed only for trials in which mice exhibited "good timing" (about 85%), that is, response onset prior to the criterion time, response offset following criterion time, and a low-high-low step function (Church *et al*., 1994). Single trial parameters were compared by using two-tailed t-test or two-way ANOVA.

Scalar property

The means of individual PI functions at each target interval were normalized on the x and y axes. The normalization yields functions centered at 1.0 (binned in units equal to 1/10 of peak time), with a height of 1.0. This permits comparison of the width of the functions independent of response-rate differences or displacement of the peak time on the ordinate. The superimposition of the functions when plotted in this manner is strong and demonstrates the scalar property (Gibbon, 1977). The coefficient of variation (CV) for each mouse was computed

as the ratio between estimation error (Peak width) and estimation accuracy (Peak time). The intra-class correlation coefficient (ICC) was calculated for each animal's normalized distribution. ICC values greater than 0.85 are indicative of excellent superposition of normalized functions for two time bins, with a value of 1.0 indicating perfect scalar timing (Buhusi *et al*., 2009). The values of CV and ICC were compared by using two-tailed t-test or two-way ANOVA.

Progressive ratio (PR) schedule

Performance in the PR task was evaluated through the Kaplan-Meier survival function (Drew *et al*., 2007). Log-rank (Mantel-Cox) test was used to determine survival differences between groups. The breaking point, the number of lever presses and the number of rewards earned were analyzed through ANOVA tests followed by post-hoc comparisons.

Catecholamine determination

Catecholamine levels in VPA and control mice were analyzed by using two-tailed t-test or two-way ANOVA.

Statistical analyses were performed using Graphpad Prism (GraphPad Software Inc., CA, USA). In all cases, the alpha level was set at 0.05.

Results

VPA treatment does not impair lever press training

During operant lever-press training, mice received 5 continuous-reinforcement schedule sessions to learn the association between lever press and food reward. There were no differences in the speed (number of sessions) with which this response was acquired ($F_{4,44}$=0.393, p=0.546 for females, n=6/group; $F_{4,84}$=1.870, p=0.173 for males, n=5-7/group, mixed-design ANOVA) nor the number of total lever presses (t_{10}=0.626, p=0.545 for females, two-tailed t-test, n=6/group; $F_{3,19}$=2.886, p=0.066, for males, one-way ANOVA, n=5-7/group).

Thus, all female and male groups acquired reliable lever pressing within 5 sessions, and there were no differences in the number of lever presses at the end of training. This result indicates that VPA mice were able to acquire operant lever press training in a similar way than controls.

VPA-VPA mice exhibit lower values of the S1 index for the short target duration during fixed-interval (FI) training

Acquisition of temporal control during FI training was evaluated using the S1 rate index in all groups for 15-s and 45-s target durations. Mean proportions of maximal response rate as a function of signal duration for FI sessions 1-3, 4-6, 7-9, 10-12 and 13-15 (3-session blocks) are illustrated in Supplementary Figures 1 and 2 for female and male VPA mice, respectively. All mice acquired temporal control of responding as a function of the elapsing signal duration across the 15 sessions of FI training. However, VPA-VPA mice presented significantly lower values of the S1 index relative to controls for the short (15-s) but not the long (45-s) target duration. The S1 rate index for the female groups is plotted as a function of session blocks in Supplementary Figures 1C and D for the FI-15s and FI-45s, respectively. Along the 5 blocks (15 FI sessions) evaluated, female VPA-VPA displayed significant differences compared to controls for the 15-s S1 index acquisition ($F_{1,29}$=5.890, p=0.041 for groups, mixed-designed ANOVA) but not for the 45-s S1 index acquisition ($F_{1,29}$=0.782, p=0.411 for groups, mixed-designed ANOVA). Supplementary Figure S2 shows the S1 rate index for the male groups. Similar to females, male VPA-VPA mice displayed significant differences compared to controls for the 15-s S1 index acquisition ($F_{3,87}$=3.753, p=0.033 for groups, mixed-designed ANOVA). Bonferroni post-hoc test indicated significant differences between VPA-VPA and SAL-SAL mice, whereas the other groups displayed values in between (Supplementary Figure 2C). There were no differences for the 45-s S1 index acquisition ($F_{3,87}$=0.808, p=0.521 for groups, mixed-designed ANOVA).

VPA-VPA mice present decreased accuracy and precision in the peak-interval (PI) task

The normalized response rate plotted as a function of signal duration for the last session block (sessions 25 to 28) of PI training is illustrated in Figures 1 and 2 for female and male groups, respectively. SAL and VPA-exposed mice acquired temporal control of responding as a function of time in probe trials, producing a Gaussian shaped response function centered close to the expected time of reinforcement. Supplementary Figure 3 exhibits examples of individual fits. Figures 1C and 2E show the main parameters from the Gaussian function (Peak time, Peak height, and Peak width). Average curve analysis indicated that female VPA-VPA mice exhibited left-shifted Peak time and diminished Peak height compared to controls for the 15-s target duration, as well as diminished Peak height and higher Peak width for the 45-s target duration (Figure 1C. 15-s target duration: t_{10}=3.408, p=0.007 for Peak time; t_{10}=3.384, p=0.007 for Peak height, and t_{10}=0.684, p=0.509 for Peak width; 45-s target duration: t_{10}=2.207, p=0.052 for Peak time; t_{10}=4.732, p=0.0008 for Peak height, and t_{10}=3.547, p=0.005 for Peak width, two-tailed t-test). Meanwhile, male VPA-VPA mice exhibited diminished Peak height and higher Peak width compared to controls for both 15-s and 45-s target durations (Figure 2E. Peak time: $F_{1,21}$=2.303, p=0.093 for groups; Peak height: $F_{1,21}$=17.09, p<0.0001 for groups; Peak width: $F_{1,21}$=6.771, p=0.001 for groups. Bonferroni post-hoc test indicated significant differences between VPA-VPA mice and the other 3 groups for both target durations. Two-way ANOVA).

Differences in timing behavior between valproic acid-exposed and control mice were also evaluated with single trial analysis. Figures 1D and 2F display the Start time, Stop time and Peak time from females and males, respectively. Female VPA-VPA mice anticipated the Start and the Peak time of the response compared to controls for the short but not the long target duration (Figure 1D, 15-s target duration: t_{10}=8.299, p=0.0002 for Peak time; t_{10}=5.993, p=0.001 for Start, and t_{10}=1.937, p=0.101 for Stop; 45-s target duration: t_{10}=0.281, p=0.788

for Peak time; t_{10}=1.636, p=0.153 for Start, and t_{10}=1.243, p=0.260 for Stop, two-tailed t-test). On the other hand, male VPA-VPA mice responded earlier in the trial compared to controls for both target durations, producing significantly lower values of Start and Peak times (Figure 2F. Peak time: $F_{1,21}$=16.63, p<0.0001 for groups; Start: $F_{1,21}$=11.28, p<0.0001 for groups. Bonferroni post-hoc test indicated significant differences between VPA-VPA mice and the other 3 groups for both target durations; Stop: $F_{1,21}$=2.195, p=0.106 for groups. Two-way ANOVA).

To further characterize the responses along PI training, the mean S1 and S2 rate indexes were used to evaluate timing precision. For both 15-s and 45-s target durations, control and VPA mice maintained the S1 and acquired a S2 response threshold (Figure 3). However, there were significant differences between SAL-SAL and VPA-VPA mice. The left panel of Figure 3 displays the mean S1 (A and B) and S2 (C and D) rate indexes from females. Along the 7 blocks (28 PI sessions) evaluated, both female groups showed stable levels of the S1 rate index across sessions (Figures 3A and B), consistent with the learning of the S1 during the FI training. The S1 rate index, however, was higher for female controls than VPA-VPA for the 15-s target duration ($F_{1,41}$=6.966, p=0.0269 for groups, $F_{6,66}$=1.330, p=0.260 for sessions; mixed-design ANOVA). There were no significant differences in the S1 rate index for the 45-s target duration ($F_{1,41}$=1.981, p=0.232 for groups, $F_{6,66}$=1.159, p=0.360 for sessions; mixed-design ANOVA). Moreover, both female groups presented an increase across sessions for the S2 rate index (Figures 3C and D), suggesting that control and VPA-VPA animals were able to learn to stop responding around the target interval. However, SAL-SAL females reached higher values of the S2 rate index across sessions for the 45-s target duration, indicating better precision compared to the VPA-VPA group (15-s: $F_{1,41}$=1.312, p=0.282 for groups,

$F_{6,66}$=6.323, p<0.0001 for sessions; 45-s: $F_{1,41}$=4.126, p=0.046 for groups, $F_{6,66}$=4.105, p=0.006 for sessions; mixed-design ANOVA).

The right panel of Figure 3 shows the mean S1 (E and F) and S2 (G and H) rate indexes from male VPA-VPA, SAL-SAL and early social stimulation groups. Similar to females, male animals showed stable levels of the S1 rate index across sessions (Figures 3E and F). There was no significant difference in the S1 rate index among groups for both 15-s and 45-s target durations (S1 15-s: $F_{3,102}$=0.154, p=0.926 for groups, $F_{6,126}$=2.482, p=0.028 for sessions. S1 45-s: $F_{3,102}$=0.866, p=0.494 for groups, $F_{6,126}$=1.147, p=0.348 for sessions; mixed-design ANOVA). On the other hand, the S2 rate index displayed an increase across sessions (Figures 3G and H), but there were significant differences among groups. VPA-exposed animals with no early social stimulation (VPA-VPA mice) displayed lower values of the S2 rate index across sessions - compared to all the other groups - for both 15-s and 45-s target durations. This result indicates that VPA-VPA males also presented worse precision along sessions compared to controls. This behavior, however, was not observed in the early social stimulation group (S2 15-s: $F_{3,102}$=5.317, p=0.0438 for groups, with Bonferroni post-hoc test indicating significant differences among VPA-VPA and the other 3 groups; $F_{6,126}$=28.94, p<0.0001 for sessions. S2 45-s: $F_{3,102}$=8.099, p=0.029 for groups, with Bonferroni post-hoc test indicating significant differences among VPA-VPA and the other 3 groups; $F_{6,126}$=7.358, p<0.0001 for sessions; mixed-design ANOVA).

VPA-VPA mice timing behavior deviates from the scalar property

We also examined the coefficient of variation (CV), the ratio between Peak width and Peak time, which is shown to be relatively constant in a large range of timed durations in several species (Buhusi & Meck, 2005). This scalar property reflects the Weber's Law applied to

interval timing, which implies superimposition of response functions when normalizing these responses to the target duration (Gibbon, 1977). Figures 4 and 5 illustrate the normalized response functions re-scaled (proportional) to the target time for female and male animals, respectively. Controls (SAL-SAL) mice (Figures 4A and 5A) presented superimposition of their response distributions for the two intervals tested (15-s and 45-s), in contrast to VPA-VPA mice (Figures 4B and 5B). Early social stimulation in VPA males yielded superimposed curves more similar to controls than to VPA-VPA mice (Figures 5C and D). Analysis of the coefficient of variation indicated that the CVs were relatively constant for the timing task in controls - thus reflecting the scalar property - but were increased in the short duration (15-s) for both females and males VPA-VPA, as shown in Figures 4C ($F_{3,30}$=10.64, p=0.005 for groups, $F_{1,21}$=7.350, p=0.015 for target intervals, two-way ANOVA) and 5E ($F_{3,30}$=4.086, p=0.0131 for groups, $F_{1,21}$=22.80, p<0.0001 for target intervals, two-way ANOVA). To further evaluate the scalar property, we then calculated the intra-class correlation coefficient, ICC, for each animal's normalized distribution. Female and male VPA-VPA mice presented significantly lower ICC values compared to controls (Figures 4D and 5F, respectively), indicating a deviation of the scalar property in these animals (Females: t_{10}=2.563, p=0.028, two-tailed t-test. Males: $F_{1,10}$=11.06, p=0.003 for growing environment, with Bonferroni post-hoc test indicating significant differences between VPA-VPA and VPA-SAL mice; $F_{1,10}$=11.41, p=0.003 for prenatal treatment. Two-way ANOVA).

VPA treatment does not alter performance in the progressive ratio (PR) schedule

We next explored whether the timing deficits observed in VPA-VPA mice might be related to their impairment in working for a food reward. Animals were evaluated in a progressive ratio (PR) schedule of reinforcement, in which the mouse is required to make an increasing

number of operant responses for each successive reward. A survival curve was obtained, in which the percentage of animals that continued responding on the PR schedule is plotted as a function of session time (Supplementary Figures 4A and D for females and males, respectively). Analysis of the survival function indicated no significant differences among groups (Chi square=2.688, p=0.101 for females, and Chi square=3.342, p=0.342 for males, Mantel-Cox test). We also examined the breaking point (number of presses for the last reward, Supplementary Figures 4B and F for females and males, respectively), as well as the total number of rewards earned (Supplementary Figures 4C and G for females and males, respectively), and the total number of lever presses made along the session (Supplementary Figures 4D and H for females and males, respectively). None of these parameters exhibited significant differences among groups (Females: breaking point t_{10}=0.521, p=0.615; # rewards earned t_{10}=0.910, p=0.386; # lever presses: t_{10}=0.601, p=0.563; two-tailed t-test. Males: breaking point $F_{1,10}$=1.049, p=0.321 for prenatal treatment, and $F_{1,10}$=0.001, p=0.971 for growing environment; # rewards earned $F_{1,10}$=0.885, p=0.361 for prenatal treatment, and $F_{1,10}$<0.001, p=0.997 for growing environment; # lever presses $F_{1,10}$=0.796, p=0.386 for prenatal treatment, and $F_{1,10}$=0.017, p=0.897 for growing environment; two-way ANOVA).

VPA-VPA mice present significant differences in striatal dopamine metabolism

In order to evaluate if the observed behavioral differences in VPA mice were related to changes in striatal catecholamine metabolism, the levels of dopamine (DA), serotonin (5-HT) and their metabolites (DOPAC and 5-HIAA, respectively) were determined from the dorsal striatum of VPA and control mice. Data for the female groups is shown in Figure 6. Female VPA-VPA mice displayed increased DA levels (Figure 6A t_{10}=2.803, p=0.019, two-tailed t-test) and decreased DA turnover (Figure 6C, t_{10}=3.053, p=0.012, two-tailed t-test) compared

to controls, while DOPAC levels were similar between groups (Figure 6B, t_{10}=0.015, p=0.989, two-tailed t-test). On the other hand, there were no significant differences between groups for 5-HT and 5-HIAA levels, as well as 5-HT turnover (Figures 6D to F, t_{10}=0.552, p=0.593 for 5-HT, t_{10}=1.274, p=0.231 for 5-HIAA, and t_{10}=1.344, p=0.2117 for 5-HT turnover, two-tailed t-test).

Figure 7 exhibits data from catecholamine determination in the male groups. Male VPA-VPA mice displayed increased DA levels compared to SAL mice growing in the same environment (SAL-VPA mice, Figure 7A, $F_{1,10}$=4.432, p=0.046 for prenatal treatment, and $F_{1,10}$=0.366, p=0.551 for growing environment, two-way ANOVA). Moreover, male VPA-VPA mice presented higher DOPAC levels and increased DA turnover compared to VPA-treated mice growing with SAL littermates (VPA-SAL mice, Figures 7B and C. DOPAC levels: $F_{1,10}$=2.093, p=0.160 for prenatal treatment, and $F_{1,10}$=22.43, p=0.003 for growing environment; DA turnover: $F_{1,10}$=0.154, p=0.698 for prenatal treatment, and $F_{1,10}$=6.146, p=0.021 for growing environment, two-way ANOVA). Similar than females, there were no significant differences between groups for 5-HT and 5-HIAA levels, as well as 5-HT turnover (Figures 7D to F, 5-HT levels: $F_{1,10}$=0.002, p=0.966 for prenatal treatment, and $F_{1,10}$=1.069, p=0.313 for growing environment; 5-HIAA levels: $F_{1,10}$=0.065, p=0.801 for prenatal treatment, and $F_{1,10}$=0.003, p=0.975 for growing environment; 5-HT turnover: $F_{1,10}$=0.367, p=0.549 for prenatal treatment, and $F_{1,10}$=1.942, p=0.174 for growing environment, two-way ANOVA).

Discussion

In the present work we evaluated the performance in the peak-interval (PI) procedure of a mouse model of prenatal exposure to valproic acid. Our results indicate that both temporal accuracy and precision are decreased in female and male VPA-VPA mice. Specifically, VPA-VPA

females exhibited a shortening in the estimation of the short duration, as indicated from both the curve average and single trial analysis (Figure 1). This left-shift in Peak time was related to earlier Start values, while no differences were observed in the Stop time. Similarly, VPA-VPA males also exhibited left-shifted Peak and Start times for both 15-s and 45-s (Figure 2), although significant differences in Peak time were only observed with single trial analysis. Moreover, a decreased Peak height and higher Peak width indicated decreased temporal precision in both female and male VPA-VPA mice (Figures 1 and 2). Indeed, data from the S1 and S2 rate indexes add more support to the notion that interval timing precision is decreased in VPA-VPA mice (Figure 3 and Supplementary Figures 1 and 2). Importantly, VPA-SAL males revealed no significant differences compared to controls (SAL-SAL), indicating that early social stimulation in male animals was able to reverse this decrement in temporal accuracy and precision. VPA prenatal treatment, on the other hand, did not affect the ability of the animals to press the lever, and the association of lever press and reward. In addition, VPA prenatally exposed animals showed a normal response to a novel object in the novel object recognition test (Lucchina & Depino 2014 and unpublished results). These findings suggest that the observed deficits in temporal processing are not related to memory deficits in these animals.

We also observed that VPA-VPA mice deviate from the scalar property of interval timing (Figures 4 and 5). These results, however, are difficult to compare to ASD in humans. While Falter *et al* (2012) reports clearer adherence to the scalar property in adults with ASD than controls in the subsecond range, Allman *et al* (2011) found less clear scalar timing among autistic children than in healthy controls in the suprasecond range. Further studies are needed in this regard. It also remains to be elucidated if the deficits in temporal processing could be related to deficits in attention in these animals. Indeed, the existing literature on interval timing does not rule out the attention component from temporal processing. On the contrary, one of the most accepted theories of interval timing, the information-processing (IP) model, includes an attentional component (e.g., Meck 1996). It has also been reported that the processes of short-time estimation and attention share similar neuroanatomic structures (Meck & Benson,

2002; Coull JT *et al.,* 2004). Therefore, future experiments could better clarify the influence of attentional components in the temporal performance of VPA mice.

Striatal catecholamine determination indicated that both female and male VPA-VPA mice presented higher DA levels than controls and significant differences in DA turnover in the dorsal striatum (Figures 6 and 7). The observed differences in DA turnover in female vs. male VPA mice (i.e., decreased DA turnover in female VPA and increased DA turnover in male VPA mice) were caused by the variation in DOPAC levels. These results are in agreement with elevated DA levels in the striatum of a mouse model of Angelman syndrome and autism (Farook *et al*., 2012). It was also reported that a larger volume of the caudate nucleus (together with an increased DRD3 expression) correlates with stereotyped autistic behavior (Staal *et al*., 2015). Also, mice carrying Pten mutations related to autism present high levels of TH and DRD2 in the striatum and prefrontal cortex (He *et al*., 2015). On the other hand, striatal serotonin metabolism was not altered in VPA-VPA mice, which is in contrast with observed alterations in the serotonergic system in humans with ASD (Nakamura *et al*., 2010). However, the number of animals used in the present study may be a limitation for the comparison of serotonin levels, and further studies are needed. Taken together, these results could explain in part the poor performance found in interval timing in VPA mice, as the literature indicates that optimum levels of DA are needed for correct temporal estimation (Coull *et al*., 2011; Jones & Jahanshahi, 2011: Agostino & Cheng, 2016). Indeed, higher striatal DA levels are in accordance with the observed left-shifted Peak and Start times in VPA-VPA mice (Soares *et al*., 2016). Consequently, our findings are related to the known mechanisms of interval timing (e.g., deficits in cortico-striatal circuits and dopaminergic status alter temporal processing).

Our results demonstrating earlier Start times (but not Stop times) in VPA-VPA mice are similar to the results obtained from a study conducted with hyperdopaminergic dopamine transporter knockdown mice (DAT KD, Balci *et al*., 2010). This study and others have pointed that leftward shifts in Peak times due to specific changes in Start times in the same direction are related to the effect of higher incentive motivation (reviewed in Balci, 2014). Other different approaches have also been reported to evaluate motivation, such as the Peak rate or Peak height in the average analysis (Ward *et al*., 2009; Yin & Meck, 2014), and the progressive ratio (PR) task. For example, alterations in the motivational state in the PR task have been shown to contribute to the observed deficits in interval timing in mice with striatal D2 receptor overexpression (Drew *et al*., 2007). Our data from the average curves indicated decreased Peak height in VPA-VPA mice, suggesting decreased motivation to respond in the PI task. However, our results from the PR task indicated no differences between groups (Supplementary Figure 4), suggesting that VPA-VPA mice do not present impairments in their motivation for food reward. Thus, the observed effects of prenatal exposure to VPA on timing behavior do not appear to be related to decreased motivation to acquire food. On the contrary, since the effect of motivation on peak responding appears to be better evaluated using single trial response-based analyses than average curve analysis (Balci, 2014), VPA mice appear to have an increased motivational state. This is consistent with the increased striatal DA levels found in these animals.

Finally, the effect of VPA exposure was more pronounced in males than females, as seen in Start and Peak times (significant differences between controls and VPA-VPA for both target durations in males and only for 15-s in females), scalar property (lower ICC values in males than females) and striatal catecholamine metabolism (DOPAC differences compared to controls in males but not in females). These findings may be in agreement with the higher risk of ASD in males (Kataoka *et al*., 2013).

Overall, our results indicate that both male and female VPA-VPA mice show significant alterations in temporal accuracy and precision - as well as in striatal dopamine metabolism - compared to control (saline) groups. These alterations were reversed after peer-rescue of autism-related behavior by early social stimulation in male mice. To our knowledge, this is the first work to describe deficits in temporal processing in a mouse model that reproduces autistic features. Our data complement previous results in humans, and provide a useful tool for further behavioral and pharmacological studies.

Acknowledgements

This work is supported by grants from the National Science Agency (ANPCyT), the National Research Council (CONICET) and the National University of Quilmes (UNQ). Supplementary data associated with this article can be found in the online version.

Conflict of Interest Statement

Authors declare no conflict of interest.

Author contributions

JA, DAG and PVA designed research. JA, MAC, HC and PVA performed experiments. JA, AMD and PVA analyzed data. JA, AMD, DAG and PVA wrote the manuscript.

Data Accessibility

All data underlying the present findings are fully available without restriction and will be shared with the research community upon request. All relevant data are within the paper.

Abbreviations

ASD, autism spectrum disorders; FI, fixed-interval; HPLC-ED; high pressure liquid chromatography coupled to electrochemical detection; LD, light-dark; PD, postnatal day; PI, peak-interval; PR, progressive ratio; SAL, saline; VPA, valproic acid; ZT, Zeitgeber time.

References

Agostino, P.V., Cheng, R.K., Williams, C.L., West, A.E. & Meck, W.H. (2013) Acquisition of response thresholds for timed performance is regulated by a calcium-responsive transcription factor, CaRF. *Genes Brain Behav,* **12**(6), 633-644.

Agostino, P.V. & Cheng, R.K. (2016) Contributions of dopaminergic signaling to timing accuracy and precision. *Curr Opin Behav Sci,* **8**, 153-160.

Agostino, P.V., Gatto, E.M., Cesarini, M., Etcheverry, J.L., Sanguinetti, A. & Golombek, D.A. (2017) Deficits in temporal processing correlate with clinical progression in Huntington's disease. *Acta Neurol Scand* doi: 10.1111/ane.12728.

Allman, M.J. & DeLeon, I.G. (2009) No time like the present: time perception in autism. In: Giordano, A.C. (Ed.). *Causes and risks for autism.* Nova Science Publishers, Inc, pp. 65-76.

Allman, M.J., DeLeon, I.G. & Wearden, J.H. (2011) Psychophysical assessment of timing in individuals with autism. *Am J Intellect Dev Disabil* **116**(2), 165-178.

Allman, M.J. & Meck, W.H. (2012) Pathophysiological distortions in time perception and timed performance. *Brain,* **135**, 656-677.

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders (DSM-5®). American Psychiatric Pub.

Balci, F., Ludvig, E.A., Abner, R., Zhuang, X., Poon, P. & Brunner, D. (2010) Motivational effects on interval timing in dopamine transporter (DAT) knockdown mice. *Brain Res,* **1325**, 89-99.

Balci, F. (2014) Interval timing, dopamine, and motivation. *Timing & Time Perception,* **2**(3), 379-410.

Belzung, C., Leman, S., Vourc'h, P. & Andres, C. (2005) Rodent models for autism: a critical review. *Drug discovery today: Disease models,* **2**(2), 93-101.

Beste, C., Saft, C., Andrich, J., Müller, T., Gold, R. & Falkenstein, M. (2007) Time processing in Huntington's disease: a group-control study. *PLoS One* **2**(12), e1263.

Boucher, J. (2001) ''Lost in a sea of time'': Time parsing and autism. In Hoerl, C. & McCormack, T. (Eds.), *Time and memory*. Oxford University Press, pp. 111–135.

Bromley, R.L., Mawer, G., Clayton-Smith, J., Baker, G.A. & Liverpool and Manchester Neurodevelopment Group (2008) Autism spectrum disorders following in utero exposure to antiepileptic drugs. *Neurology* **71**(23), 1923-1924.

Buhusi, C.V. & Meck, W.H. (2005) What makes us tick? Functional and neural mechanisms of interval timing. *Nat Rev Neurosci,* **6**, 755-765.

Buhusi, C.V., Aziz, D., Winslow, D., Carter, R.E., Swearingen, J.E. & Buhusi, M.C. (2009) Interval timing accuracy and scalar timing in C57BL/6 mice. *Behav Neurosci,* **123**(5), 1102-1113.

Bussi, I.L., Levin, G., Golombek, D.A. & Agostino, P.V. (2014) Involvement of dopamine signaling in the circadian modulation of interval timing. *Eur J Neurosci,* **40**(1), 2299-2310.

Bussi, I.L., Levin, G., Golombek, D.A. & Agostino, P.V. (2015) Melatonin modulates interval timing in rats: effect of pinealectomy. *Int J Comp Psychol,* **28**(1).

Cheng, R.K. & Meck, W.H. (2007) Prenatal choline supplementation increases sensitivity to time by reducing non-scalar sources of variance in adult temporal processing. *Brain Res,* **1186**, 242-254.

Church, R.M., Meck, W.H. & Gibbon, J. (1994) Application of scalar timing theory to individual trials. *J Exp Psychol Anim Behav Process,* **120**(2), 135-155.

Coull, J.T., Vidal, F., Nazarian, B., & Macar, F. (2004) Functional anatomy of the attentional modulation of time estimation. *Science,* **303**(5663), 1506-1508.

Coull, J.T., Cheng, R.K. & Meck, W.H. (2011) Neuroanatomical and neurochemical substrates of timing. *Neuropsychopharmacology,* **36**, 3-25.

Drew, M.R., Simpson, E.H., Kellendonk, C., Herzberg, W.G., Lipatova, O., Fairhurst, S., Kandel, E.R., Malapani, C. & Balsam, P.D. (2007) Transient overexpression of striatal D2 receptors impairs operant motivation and interval timing. *J Neurosci,* **27**(29), 7731-7739.

Dufour-Rainfray, D., Vourc'h, P., Tourlet, S., Guilloteau, D., Chalon, S. & Andres, C.R. (2011) Fetal exposure to teratogens: evidence of genes involved in autism. *Neurosci Biobehav Rev* **35**(5), 1254-1265.

Falter, C.M., Noreika, V., Wearden, J.H. & Bailey, A.J. (2012) More consistent, yet less sensitive: interval timing in autism spectrum disorders. *Q J Exp Psychol (Hove),* **65**(11), 2093-2107.

Farook, M.F., DeCuypere, M., Hyland, K., Takumi, T., LeDoux, M.S. & Reiter, L.T. (2012) Altered serotonin, dopamine and norepinepherine levels in 15q duplication and Angelman syndrome mouse models. *PLoS One,* **7**(8), e43030.

Gibbon, J. (1977) Scalar expectancy theory and Weber's Law in animal timing. *Psychol Rev,* **84**, 279-325.

He, X., Thacker, S., Romigh, T., Yu, Q., Frazier, T.W. Jr & Eng, C. (2015) Cytoplasm-predominant Pten associates with increased region-specific brain tyrosine hydroxylase and dopamine D2 receptors in mouse model with autistic traits. *Mol Autism*, **6**, 63.

Högl, B., Agostino, P.V., Peralta, M.C., Gershanik, O. & Golombek, D.A. (2014) Alterations in time estimation in multiple system atrophy. *Basal Ganglia*, **4**(3), 95-99.

Jones, C.R. & Jahanshahi, M. (2011) Dopamine modulates striato-frontal functioning during temporal processing. *Front Integr Neurosci,* **5**, 70.

Kataoka, S., Takuma, K., Hara, Y., Maeda, Y., Ago, Y. & Matsuda, T. (2013) Autism-like behaviours with transient histone hyperacetylation in mice treated prenatally with valproic acid. *Int J Neuropsychopharmacol,* **16**, 91-103.

Kazlauskas, N., Campolongo, M., Lucchina, L., Zappala, C. & Depino, A.M. (2016) Postnatal behavioral and inflammatory alterations in female pups prenatally exposed to valproic acid. *Psychoneuroendocrinology,* **72**, 11-21.

Kim, K.C., Kim, P., Go, H.S., Choi, C.S., Park, J.H., Kim, H.J., Jeon, S.J., Han, S.H., Cheong, J.H., Ryu, J.H. & Shin, C.Y. (2013) Male-specific alteration in excitatory post-synaptic development and social interaction in pre-natal valproic acid exposure model of autism spectrum disorder. *J Neurochem,* **124**, 832-843.

Kim, K.C., Kim, P., Go, H.S., Choi, C.S., Yang, S.I., Cheong, J.H., Shin, C.Y. & Ko, K.H. (2011) The critical period of valproate exposure to induce autisticsymptoms in Sprague-Dawley rats. *Toxicology Letters,* **201**(2), 137-142.

Kim, Y.S., Leventhal, B.L., Koh, Y.J., Fombonne, E., Laska, E., Lim, E.C., Cheon, K.A., Kim, S.J., Kim, Y.K., Lee, H., Song, D.H. & Grinker, R.R. (2011) Prevalence of autism spectrum disorders in a total population sample. *Am J Psychiatry,* **168**, 904-912.

Lucchina, L. & Depino, A.M. (2014) Altered peripheral and central inflammatory responses in a mouse model of autism. *Autism Res,* **7**(2), 273-289.

Lustig, C., Matell, M.S. & Meck, W.H. (2005) Not "just" a coincidence: Frontal-striatal synchronization in working memory and interval timing. *Memory,* **13**, 441-448.

Malapani, C., Rakitin, B., Levy, R., Meck, W.H., Deweer, B., Dubois, B. & Gibbon, J. (1998) Coupled temporal memories in Parkinson's disease: A dopamine-related dysfunction. *J Cogn Neurosci,* **10**, 316-331.

Mandy, W. & Lai, M.C. (2016) Annual Research Review: The role of the environment in the developmental psychopathology of autism spectrum condition. *J Child Psychol Psychiatry*, **57**(3), 271-292.

Marquardt, D.W. (1963) An algorithm for least-squares estimation of nonlinear parameters. *J Soc Indust Appl Math,* **11**(2), 431-441.

Martin, J.S., Poirier, M. & Bowler, D.M. (2010) Brief report: Impaired temporal reproduction performance in adults with autism spectrum disorder. *J Autism Dev Disord,* **40**(5), 640-646.

Matell, M.S., Meck, W.H. & Nicolelis, M.A.L. (2003) Interval timing and the encoding of signal duration by ensembles of cortical and striatal neurons. *Behav Neurosci,* **117**, 760-773.

Matell, M.S., Bateson, M. & Meck, W.H. (2006) Single-trials analyses demonstrate that increases in clock speed contribute to the methamphetamine-induced horizontal shifts in peak-interval timing functions. *Psychopharmacology,* **188**(2), 201-212.

Meck, W.H. (1996). Neuropharmacology of timing and time perception. *Cognitive Brain Research*, **3**, 227–242.

Meck, W.H., & Benson, A.M. (2002) Dissecting the brain's internal clock: how frontal-striatal circuitry keeps time and shifts attention. *Brain and Cognition*, **48**(1), 195-211.

Meck, W.H. (Ed.). (2003) *Functional and neural mechanisms of interval timing*. CRC Press.

Moore, S.J., Turnpenny, P., Quinn, A., Glover, S., Lloyd, D.J., Montgomery, T. & Dean, J.C. (2000) A clinical study of 57 children with fetal anticonvulsant syndromes. *J Med Genet*, **37**, 489-497.

Nakamura, K., Sekine, Y., Ouchi, Y., Tsujii, M., Yoshikawa, E., Futatsubashi, M. & Matsuzaki, H. (2010) Brain serotonin and dopamine transporter bindings in adults with high-functioning autism. *Arch Gen Psychiatry,* **67**(1), 59-68.

Patterson, H.P. (2011) Modeling autistic features in animals. *Pediatr Res,* **69**(5 Pt 2), 34R–40R.

Richardson, N.R. & Roberts, D.C. (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Methods*, **66**, 1-11.

Schneider, T. & Przewłocki, R. (2005) Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology* **30**(1), 80-89.

Schneider, T., Roman, A., Basta-Kaim, A., Kubera, M., Budziszewska, B., Schneider, K. & Przewłocki, R. (2008) Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. *Psychoneuroendocrinology,* **33**, 728-740.

Soares, S., Atallah, B.V. & Paton, J.J. (2016) Midbrain dopamine neurons control judgment of time. *Science,* **354**(6317), 1273-1277.

Staal, W.G., Langen, M., van Dijk, S., Mensen, V.T. & Durston, S. (2015) DRD3 gene and striatum in autism spectrum disorder. *Br J Psychiatry*, **206**(5), 431-432.

Stromland, K., Nordin, V., Miller, M., Akerstrom, B. & Gillberg, C. (1994) Autism in thalidomide embryopathy: a population study. *Dev Med Child Neurol,* **36**, 351-356.

Szelag, E., Kowalska, J., Galkowski, T. & Pöppel, E. (2004) Temporal processing deficits in high-functioning children with autism. *Br J Psychol,* **95**(3), 269-282.

Wagner, G.C., Reuhl, K.R., Cheh, M., McRae, P. & Halladay, A.K. (2006) A new neurobehavioral model of autism in mice: Preandpostnatal exposure to sodium valproate. *J Autism Dev Disord,* **36**, 779-793.

Ward, R.D., Kellendonk, C., Simpson, E.H., Lipatova, O., Drew, M.R., Fairhurst, S., Kandel, E.R. & Balsam, P.D. (2009) Impaired timing precision produced by striatal D2 receptor overexpression is mediated by cognitive and motivational deficits. *Behav Neurosci,* **123**(4), 720-730.

Yang, M., Perry, K., Weber, M.D., Katz, A.M. & Crawley, J.N. (2011) Social peers rescue autism-relevant sociability deficits in adolescent mice. *Autism Res,* **4**, 17-27.

Yin, B. & Meck, W.H. (2014) Comparison of interval timing behaviour in mice following dorsal or ventral hippocampal lesions with mice having δ-opioid receptor gene deletion. *Philos Trans R Soc Lond B Biol Sci,* **369**(1637), 20120466.

Zablotsky, B., Black, L.I., Maenner, M.J., Schieve, L.A. & Blumberg, S.J. (2015) Estimated Prevalence of Autism and Other Developmental Disabilities Following Questionnaire Changes in the 2014 National Health Interview Survey. *Natl Health Stat Report*, **87**, 1-20.

Figure captions

Figure 1. Peak interval (PI) timing in female mice. Normalized response rate of PI trials as a function of time in the trial for (A) female SAL-SAL and (B) female VPA-VPA mice during the last 4-session block (sessions 25-28) of PI training. Dashed lines indicate target times (15-s and 45-s). (C) Mean best-fit parameter values from the Gaussian fits: Peak time (upper panel), Peak height (middle panel), and Peak width (bottom panel). (D) Mean values from single trial analysis: Peak time (upper panel), Start (middle panel), and Stop (bottom panel). Data are shown as mean ± SEM, n=6/group. ***p<0.001, **p<0.01, *p<0.05, two-tailed t-test.

Figure 2. Peak interval (PI) timing in male mice. Normalized response rate of PI trials as a function of time in the trial for (A) male SAL grown in SAL environment (SAL-SAL), (B) male VPA grown in VPA environment (VPA-VPA), (C) male SAL grown in VPA environment (SAL-VPA, low sociability reversion controls), and (D) male VPA grown in SAL environment (VPA-SAL, low sociability reversion) during the last 4-session block (sessions 25-28) of PI training. Dashed lines indicate target times (15-s and 45-s). (E) Mean best-fit parameter values from the Gaussian fits: Peak time (upper panel), Peak height (middle panel), and Peak width (bottom panel). (F) Mean values from single trial analysis: Peak time (upper panel), Start (middle panel), and Stop (bottom panel). Data are shown as mean ± SEM, n=7 for SAL-SAL and n=5 for the other groups. Asterisks denote significant differences between VPA-VPA mice and the rest of the groups. ***p<0.001, **p<0.01, *p<0.05, two-way ANOVA followed by Bonferroni post-hoc test.

Figure 3. Acquisition of S1 and S2 rate indexes during peak-interval (PI) training. The left panel shows the mean S1 (A and B) and S2 (C and D) rate indexes across sessions for either SAL-SAL (circles) or VPA-VPA (squares) female mice. The right panel exhibits the mean S1 (E and F) and S2 (G and H) rate indexes across sessions for either SAL-SAL (white circles), VPA-VPA (dark

circles), SAL-VPA (white squares) and VPA-SAL (dark squares) male mice. Sessions are plotted as blocks of 4 sessions each. Data are shown as mean ± SEM. *p<0.05, mixed-design (two-way repeated measures) ANOVA.

Figure 4. Scalar property in female mice. Normalized response distributions plotted as a function of relative time for (A) female SAL-SAL and (B) female VPA-VPA mice. (C) Coefficient of variation (CV) across target intervals for SAL-SAL (circles) and VPA-VPA (squares) mice. Data are shown as mean ± SEM. (D) Scatter plot of the intraclass correlation coefficient (ICC) including mean ± SEM for SAL-SAL (circles) and VPA-VPA (squares) mice, n=6/group. CV: **p<0.01, two-way ANOVA. ICC: *p<0.05, two-tailed t-test.

Figure 5. Scalar property in male mice. Normalized response distributions plotted as a function of relative time for (A) male SAL grown in SAL environment (SAL-SAL), (B) male VPA grown in VPA environment (VPA-VPA), (C) male SAL grown in VPA environment (SAL-VPA, low sociability reversion controls), and (D) male VPA grown in SAL environment (VPA-SAL, low sociability reversion). (E) Coefficient of variation (CV) across target intervals for SAL-SAL (clear circles), VPA-VPA (filled squares), SAL-VPA (filled circles), and VPA-SAL (clear squares). Data are shown as mean ± SEM. (F) Scatter plot of the intraclass correlation coefficient (ICC) including mean ± SEM, n=7 for SAL-SAL and n=5 for the other groups. *p<0.05, two-way ANOVA followed by Bonferroni post-hoc test.

Figure 6. Striatal catecholamine levels in female mice. DA, 5-HT and their metabolites (DOPAC and 5-HIAA, respectively) were measured by HPLC-ED. Samples were taken at Zeitgeber time (ZT) 18 from SAL and VPA female mice. (A) DA, (B) DOPAC, (C) DA turnover, (D) 5-HT, (E) 5-HIAA, and (F) 5-HT turnover. Data are expressed as scatter plots including mean ± SEM, n=6/group. *p<0.05, two-tailed t-test.

Figure 7. Striatal catecholamine levels in male mice. DA, 5-HT and their metabolites (DOPAC and 5-HIAA, respectively) were measured by HPLC-ED. Samples were taken at Zeitgeber time (ZT) 18 from SAL-SAL, VPA-VPA, SAL-VPA and VPA-SAL male mice. (A) DA, (B) DOPAC, (C) DA turnover, (D) 5-HT, (E) 5-HIAA, and (F) 5-HT turnover. Data are expressed as scatter plots including mean ± SEM, n=7 for SAL-SAL and n=5 for the other groups. **p<0.01, *p<0.05, two-way ANOVA followed by Bonferroni post-hoc test.