Exp Brain Res (2017) 235:2787–2796
DOI 10.1007/s00221-017-5014-4

RESEARCH ARTICLE

Aberrant connections between climbing fibres and Purkinje cells induce alterations in the timing of an instrumental response in the rat

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Received: 14 February 2017 / Accepted: 15 June 2017 / Published online: 20 June 2017

Abstract Cerebellar participation in timing and sensory-motor sequences has been supported by several experimental and clinical studies. A relevant role of the cerebellum in timing of conditioned responses in the range of milliseconds has been demonstrated, but less is known regarding the role of the cerebellum in supra-second timing of operant responses. A dissociated role of the cerebellum and striatum in timing in the millisecond and second range had been reported, respectively. The climbing fibre-Purkinje cell synapse is crucial in timing models; thus, the aberrant connection between these cellular elements is a suitable model for evaluating the contribution of the cerebellum in timing in the supra-second range. The aberrant connection between climbing fibres and Purkinje cells was induced by administration of the antagonist of NMDA receptors MK-801 to Sprague–Dawley rats at postnatal days 7–14. The timing of an operant response with two fixed intervals (5 and 8 s) and egocentric sequential learning was evaluated in 60-day-old adult rats. The aberrant connections caused a reduced accuracy in the timing of the instrumental response that was more evident in the 8-s interval and a reduced number of successive correct responses (responses emitted in the correct second without any other response between them) in the 8-s interval. In addition, an inability to incorporate new information in a sequence previously learned in egocentric-based sequence learning was apparent in rats with aberrant CF–PC synapses. These results support a relevant role for the cerebellum in the fine-tuning of the timing of operant responses in the supra-second range.

Keywords Cerebellum · Climbing fibres · Timing · VGlut2 · Purkinje cell

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Introduction

Timing is a term used to describe the processes of estimating the duration of stimuli or events, as well as the predicting the occurrence of a stimulus and its relative position among a series of stimuli or events. Studies of timing can assess the estimation of the duration of a stimulus or an inter stimulus interval (estimating the interval durations) in a passive form or through motor acts. The subjects can express the duration of one stimulus or inter stimulus interval through the emission of one sustained, delayed or periodic motor response. Additionally, timing encompasses estimations or predictions about "When" an event is probably to occur and judgements of temporal order (Coull et al. 2011).

Studies have related the cerebellum with timing, showing a strong relation between cerebellar activity and temporal processing in the sub-seconds range in several paradigms both in animal models and in human beings. Neuroimaging studies have revealed the cerebellar participation in sensory-motor timing. For instance,

during repetition and continuation of tapping in humans, increased cerebellar activation is observed ipsilateral to the hand used by the participants (Jueptner et al. 1995). Additionally, in tasks in which the participants must synchronize their movements to a metronome, a bilateral activation of the cerebellum was observed (Jantzen et al. 2005), whereas there is a bilateral cerebellar activation when temporal patterns must be held through a delay (Bengtsson et al. 2005). In addition, Imaging studies have revealed the cerebellar participation in gross temporal discrimination in a task where human participants judged the duration of the second tone of a pair (Tregellas et al. 2006). Furthermore, patients with cerebellar lesions show impairments in discriminating short and long intervals (400 ms to 4 s) (Mangels et al. 1998) and are impaired in the production of intervals as long as 10 s, although their perception of the intervals was unimpaired (Gooch et al. 2010).

However, experimental studies principally focus on timing in the range of milliseconds and in classical conditioning rather than voluntary movements. In this sense, the primary role for the cerebellum in the range of 50–500 ms has been observed in eye blink conditioning, in animal models (Christian and Thompson 2003; Mauk and Donegan 1997; Kehoe 2006). Additionally, it was shown that the cerebellar cortex participated in learning of temporal information, whereas the interpositus nucleus (IN) was related to the expression of timed behaviour in eyelid conditioning tasks in rabbits (Ohyama and Mauk 2001). Moreover, Callu et al. (2009) evaluated the effect of IN lesions in a temporal discrimination task on the order of 2 and 8 s in rats and observed a reduced temporal discrimination that was overridden with training.

In humans the selective activation of the inferior olive (IO) monitored by functional magnetic resonance imaging (FMRI) was reported during the perception of complex temporal sequences of visual stimuli, and during a task of perception of the change of stimulus timing, in contrast to attention to timing only (Liu et al. 2008; Xu et al. 2006). Information from the IO arrives to the cerebellum through the climbing fibres (CF), and their contacts on the Purkinje cells (PC) are considered to be a principal component in the cerebral timing system (Jacobson et al. 2008; Yarom and Cohen 2002). Climbing fibres make contact with the proximal dendritic field of PCs where they exert a strong excitatory effect (Strata and Rossi 1998). In early postnatal days, the cerebellum shows multiple CF contacts (from many IO cells) on each PC, but after the maturation process takes place and the adult cerebellum presents a one to one relation between CF and PC (Crepel et al. 1976; Eccles et al. 1966; Hashimoto and Kano 2005). The critical period of synaptic elimination includes a two-stage process from P7 to P17 (Hashimoto and Kano 2013).

Application of the antagonist of NMDA receptors MK-801 on postnatal days 15 and 16 in mice induces the connection of multiple CFs on a PC, as well as aberrant contacts (contacts on the upper dendritic tree of the PC where the normal adult cerebellar cortex does not possess CF contacts) and concomitantly motor alterations on a rota-rod task in the adult mice. In this task, the control mice were able to remain on the rota-rod (rotating at 8 rpm) for 120 s, whereas experimental mice remained only half of that time (Kakizawa et al. 2000). Thus, induction of multiple and aberrant connections between CFs and PCs is a suitable model to assess the contribution of CF–PC synapses to timing. With the hypothesis that, similar to their effect on the motor function, the aberrant connection between CF and PC will disrupt the timing ability of the rats, in this work, MK-801 was administered to rats on postnatal days 7–14 and supra-seconds timing was assessed in the adult rats. The rats learned to emit an operant response using continuous reinforcement schedules with fixed intervals of 5 and 8 s. In addition, a sequential egocentric-based task was applied to the rats to assess the effect of CF–PC aberrant connections in motor sequence learning; a deficient egocentric sequential ability was expected to occur in the MK-801-treated rats, due to the role of the cerebellum in the learning of sequential events (Gaytan-Tocaven and Olvera-Cortes 2004; Hikosaka et al. 1998; Lu et al. 1998; Molinari et al. 1997).

Methods

Animals

Experimental procedures were performed in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23). Experimental procedures were approved by the Research and Ethics Committee of the Instituto Mexicano del Seguro Social, México.

Thirty-four Sprague–Dawley male rats maintained under facility conditions were included in the study. One experimental group (EXP, $n = 12$) received a daily intraperitoneal injection of MK-801 (0.25 μg/g of body weight from a solution of 15 μg/ml, in saline solution) from 7 to 14 postnatal days when the elimination of multiple contacts of CF on PC in the molecular layer of rats occurs (Hashimoto and Kano 2013).

One control group (CTR, $n = 8$) received a vehicle solution at similar volumes and days as the EXP group. The rats were weaned, maintained and housed in groups until reaching the age of 2 months (day postnatal 60) when the behavioural experiments began. The rats were trained in a Skinner box using two counterbalanced fixed interval schedules

(5 and 8 s), and in an egocentric sequential learning task using a plus maze with high walls; the order of the training in the tests was counterbalanced for the animals of the groups. Once the behavioural experiments were finalized, the rats were intracardially perfused with a washing solution (phosphate buffer) and a fixating solution (para-formaldehyde), and the cerebellum was extracted and stored in a cryoprotector solution.

Additionally, 10 more rats divided into two groups were used as controls for the changes induced by training; five rats received vehicle solution in similar treatment as the CTR group, and five rats received MK-801 in similar treatment to the EXP group. These two groups of rats were intracardially perfused at postnatal day 60 without any behavioural training. The cerebellum of the four groups of rats was extracted and sliced (30 μm) in the cryostat, and the coronal slices were submitted to the immunohistochemical procedure for immunostaining of VGlut2.

Behavioural tests

Sequential task

An elevated plus maze with high walls (40 cm) in the four arms (50 cm × 12 cm) was used for the sequential egocentric task. The rats were deprived of food (80% of the daily requirement) 1 week before the training during which fruit cereal was used as reinforcement. The rats were placed into the maze for 10 min with all arms opened and baited during two consecutive days of habituation. After habituation, 7 days of forced training was given to the rats as follows: the sequence started with placing the rat in one arm of the maze (randomly chosen) with the door on the arm raised and the arm to the right of the starting arm was the only other door opened. When the rat found the pellet, the arm was closed and was considered the new starting arm. After the rat consumed the cereal and returned to the door of the closed arm, the door was raised and the left arm was the only other door open. Once the rat entered the arm of the left, the door was closed and the arm was again the starting arm for the following trial of the sequence. The other two arms of the sequence were the left and the front arm. Thus, the entire sequence was: right–left–left–front (Fig. 1); two daily forced sequences were applied to the rats. After 7 days of forced training, the rats were given seven more days under free choice condition. In these days, the four arms of the maze were available for the rats, and two daily free choice trials were applied to the rats. In these sequences, the number of entries before locating the correct arm (errors), or until 5 min elapsed, was registered, and the mean number of errors from the two daily sequences was compared between both intra- and inter-groups. Additionally, a score for the number of correct sequential entries to the arms was calculated (sequential score, SS) valuing with 1 point for each correct entry in a sequence and 1/number of errors, for each incorrect entry. With this procedure, a rat without errors had a score of four in one sequence (four arms chosen in the correct order), a rat with three arms chosen in correct order and eight errors in one arm election (eight total errors) had a SS value of 3.125, whereas a rat with two errors in each arm election (eight total errors) had a SS value of 2.0. Finally, a rat with two correct sequential entries and six errors in one entry and two errors in the other entry had a SS of 2.66 (with eight total errors). Thus, it is evident that rats with a similar number of errors had different value of sequential score, depending on the sequential correctly remembered entries. The mean of the SS obtained in the two sequences each day was obtained, and intra- and inter-group comparisons were made with blocks of 2 days for both the number of errors and SS. The maze was cleaned and the odour was equalized through the maze, both between each sequence and between each rat.

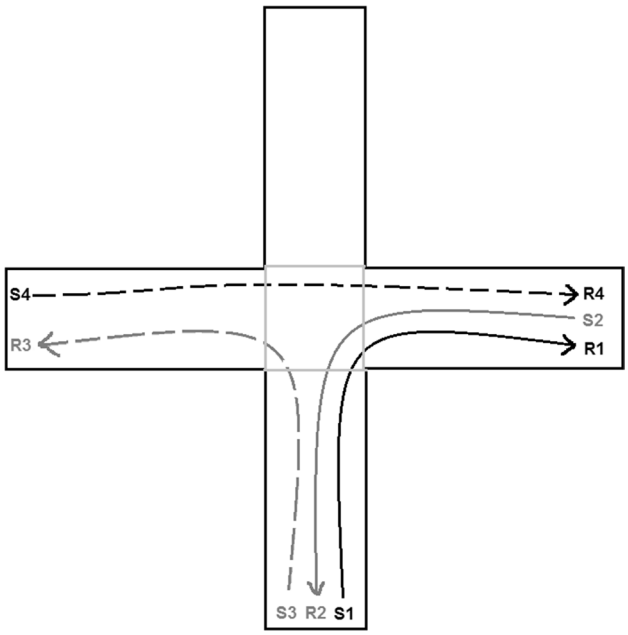


Fig. 1 Schematic representation of the plus maze and the sequence of rewarded arms. *S1–S4*, starting arms; *R1–R4*, rewarded arms. The *square* in the centre of the maze indicates the guillotine doors. The total sequence was right–left–left–front. The *S1* arm for each trial (two trials were performed per rat each day) was randomly chosen

Timing

The rats were habituated to the operating chamber for 2 days for 10 min each day. On the third day, the rats were submitted to water restriction and trained to press the lever to obtain one drop of water using a continuous-reinforcement schedule for 5 days (50 reinforced responses each day) in which

the rats developed the instrumental response; although the rats were free to run around the cage, in this period of pre-training they learned to remain next to the panel with the light and the dispenser of water. Following, a fixed-interval schedule began (5 or 8 s counterbalanced between the rats from the two groups); the trial initiated when the rats press the lever for the first time, a light in the front panel was switched on and a drop of water was delivered, following, the rats were rewarded only when they pressed the lever in the (5ht or 8ht) second after the previous correct press of the lever. The light in the panel above the lever was switched on whenever the rat pressed the lever in the correct second, and the light remained switched on during the rewarded second (5 or 8) and during the next second (6 and 9). When the rat failed to press in the correct second, the clock continues running and the reward was delivered to the rat if pressed the lever in the next correct second. This usually occurred since the rats remained pressing the lever until receiving some drop of water, initially pressing in a continuous manner, and after pressing with higher rate in the rewarded seconds.

The training occurred over 20 days, and the rats were maintained in the operant chamber each day until they reached 50 correct responses. The percentage of responses for each second and day was counted and compared. Also the number of successive correct responses (successive responses occurred exactly each 5 or 8 s without any other response between them) was counted and compared. This measurement was realized because we observed through the training that the rats pressed the lever initially at each second and then concentred on the press of the lever around the second rewarded, to finally begin to show sequences of pressing of lever each 5 or 8 s without any other response through the interval. This last timing can be considered as a better index of the ability of the rats to measure the exact time interval to receive the reward, and as a fine-tuning of the timing in this task.

Immunohistochemistry

The cerebellum was sliced in a cryostat and the slices were processed for the immunostaining of Vglut2, which is restricted to the CF synaptic buttons in the cerebellar molecular layer of adult rats (Fremeau et al. 2001). Immunostaining was performed using a primary monoclonal antibody anti-VGLUT 2 and a secondary polyclonal antibody anti-rabbit, coupled with Texas-red (CHEMICON®). Once immunostained, five sagittal slices for each rat were chosen, and two rectangular areas of 300 μm^2 were marked, covering the total extension of the molecular layer, per slice. The number of immunoreactive marks dividing the field in the proximal and distal halves of the total length of the molecular layer extension was counted. In addition, the percentage of the extension of the climbing fibres was measured, considering the total length of the molecular layer as 100%. The measures were made using the software AXOVISION LE 4.5. The number of immunoreactive marks in the proximal and distal fields and the percentage of the extension of CF in the molecular layer were obtained; the means per rat were used in an ANOVA with two factors: group and field, and *t* tests with Bonferroni correction were used for post hoc analysis.

Results

Immunohistochemistry

The administration of MK-801 on P7–P14 induced aberrant contacts of CFs on PCs in the cerebellum of adult rats. Figure 2 shows a microphotography array of the folia of the cerebellar cortex immunostained for Vglut 2. The mean number of immunoreactive marks of the proximal and distal fields and the percentage of the extension of the immunoreactive marks with regard to the total length of the molecular layer were compared between the four groups of rats (two groups receiving MK801, one trained and one untrained, and two CTR groups, one trained and one untrained). There were no differences in the distal and proximal immunoreactive marks between the trained and untrained CTR rats [$F(3, 25) = 3.489$, $p = 0.032$; paired comparisons not significant] or between trained and un-trained EXP rats [$F(3, 23) = 0.9642$; $p = 0.428$]. Thus, the trained and un-trained groups were collapsed and compared between groups (Fig. 2, bottom). The interaction of group and field (proximal and distal) was significant [$F(3,49) = 4.369$, $p = 0.008$] and paired comparisons showed a smaller number of immunoreactive VGlut2 marks for the CTR distal field compared with the CTR proximal field ($p = 0.0012$) and higher number of marks in the EXP distal field compared with the CTR distal field ($p = 0.005$). In addition, the percentage of the extension of immunoreactive VGlut2 marks was higher for the EXP group compared with the CTR group (two-tailed $t = 8.772$, $df = 23$, $p < 0.0001$) (Fig. 2 bottom). Thus, aberrant connections between CFs and PCs were evident in the increased density in the distal half of the dendritic tree as well as the increased extension of synaptic buttons in the more distal dendritic tree of the PCs (Fig. 2).

Behaviour

Egocentric sequential learning

Blocks of 2 days of errors were compared with the number of errors committed by the rats the first day of training (ANOVA for Block design, and Tukey's test). There were no changes through the blocks of training days in the CTR group [$F(3,$

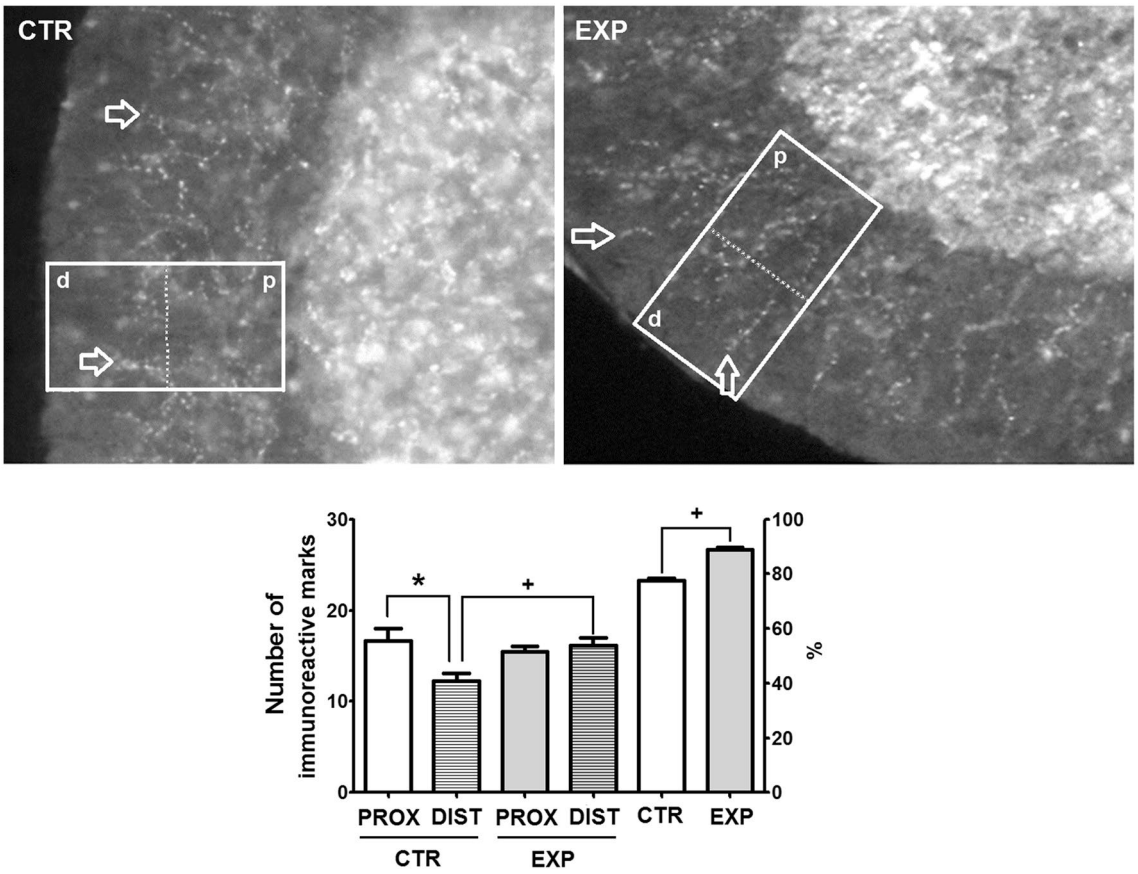


Fig. 2 *Top* microphotography of one CTR and one EXP slice of the cerebellar folia immunostained for VGlut2. *Arrows* indicate the limit of the presence of VGlut2 immunoreactive marks. Magnification 20×. *Bottom* number of VGLUT2 immunoreactive marks in the proximal and distal fields of one rectangular area of 300 μm^2 encompassing all of the molecular layer of the cerebellar folia for the EXP and CTR groups (*left Y-axis*). Percentage of the extension of VGlut2 immunoreactive marks considering the total length of the molecular layer as 100% (*right Y-axis*). Mean ± SE. *, Proximal vs. distal; +, CTR vs. EXP. $p < 0.05$

33) = 1.617, $p = 0.204$]; or for EXP group [$F(3, 57) = 0.186$, $p = 0.906$]. Between-group comparisons did not show significant differences by group [$F(1, 14) = 0.165$, $p = 0.691$] or by group and block [$F(3, 42) = 0.889$, $p = 0.455$]. In the sequential score (SS) there were no significant differences through the blocks of training days in the CTR group [$F(3, 33) = 1.831$, $p = 0.161$] or in the EXP group [$F(3, 57) = 0.136$, $p = 0.938$]. However, there were differences in the SS when comparing group and block of training [$F(3, 45) = 2.831$, $p = 0.049$]. Paired comparisons showed a higher SS for the EXP group in block 1 ($p = 0.036$) and a bias in block 2 ($p = 0.054$) compared with the CTR group (Fig. 3).

Timing of an instrumental response

The percentage of correct responses in the 8- and 5-s fixed intervals through blocks of 4 days were compared in both groups (two-way ANOVA, with factors being block and second).

Intra-group comparison of the percentage of responses in the 5-s interval did not show a significant effect for the interaction between block and second for the CTR group [$F(20, 775) = 1.125$, $p = 0.317$]. The CTR rats exhibited a higher number of responses in second 5 after the first block of training, but no further increase was observed with the elapse of the training blocks (Fig. 4a). The EXP group showed a significant effect of the block and second factor interaction [$F(20, 1399) = 3.504$, $p < 0.001$]; the percentage of responses was higher in the rewarded second (second 5) in blocks 4 ($p = 0.0002$) and 5 ($p = 0.0001$) (Fig. 4b) compared with the first, whereas a lower number of responses occurred in the second 6 on block 5 ($p = 0.041$), and a lower number of responses in the second 1 on blocks 4 ($p = 0.045$) and 5 (0.014). Thus, the EXP group showed a gradual increase throughout training until reaching a maximal performance and increased the number of responses with the training days only in the rewarded second.

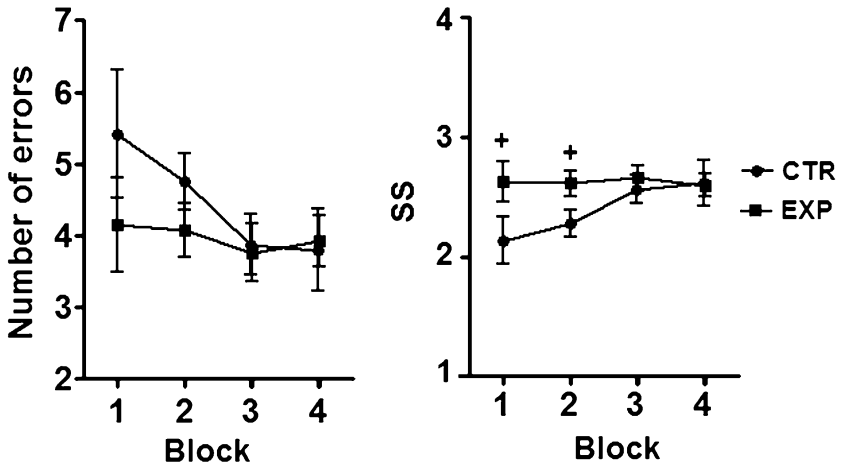


Fig. 3 Number of errors and *SS* of the *CTR* and *EXP* groups in the egocentric-based sequence learning. Mean ± SE. Blocks of 2 days (b2–b4) were compared with day 1 of training (b1). +, *CTR* vs. *EXP*. $p < 0.05$

In the intra-group comparisons for the 8-s interval, the CTR group showed a significant effect of the training block on the number of responses per second [$F(32, 1250) = 2.321$, $p < 0.001$]; paired comparisons showed an increased number of correct responses for second 8 in blocks 3 ($p = 0.0005$), 4 ($p = 0.0002$) and 5 ($p < 0.0001$) compared with the first block (Fig. 4a, b). The EXP group also showed a significant effect of the training block [$F(32, 2104) = 4.495$, $p < 0.001$]; paired comparisons showed not only an increased number of responses for second 8 in blocks 2 ($p = 0.030$), 3 ($p = 0.0011$), 4 and 5 ($p < 0.0001$) but also an increased number of responses for second 7 in blocks 3, ($p = 0.0049$), 4 ($p = 0.0037$) and 5 ($p < 0.0001$). Thus, EXP group showed increased responses for both seconds 7 and 8, showing a lower precision in the detection of the rewarded second. However, no significant interaction of group, block and second was observed for the 5- or 8-s interval (Fig. 4a, b).

In the comparison of successive correct responses (sequences of responses in the rewarded second, without any other response in the interval), the CTR group showed increased sequential correct responses through training in the 8-s interval [$F(4,159) = 3.822$, $p = 0.005$]; blocks 4 ($p = 0.0197$) and 5 ($p = 0.0011$) had a significantly higher number of cumulated successive correct responses compared with block 1. This increase was absent in the EXP group [$F(4, 239) = 0.3685$, $p = 0.83$). Inter-group comparisons showed a higher number of sequential responses in the CTR group [$F(1, 78) = 37.17$, $p < 0.001$, main effect; (two-tailed $t = 8.001$, $df = 398$, $p < 0.0001$)], whereas the interaction of group and block was not significant. There was no increase in the successive correct responses through training in the 5-s interval for any group, and no inter-group differences existed (Fig. 4c).

Discussion

Kakizawa et al. (2000) showed the dependence of the climbing fibres synaptic elimination process, on NMDA receptors in the cerebellar molecular strata, through the administration of the NMDA receptor antagonist MK-801 on P15 and P16; the authors observed aberrant connections of climbing fibres on Purkinje cells of adult mice. These mice did not show obvious gross motor dysfunction, but showed a mild motor alteration.

In the present work, MK-801 was administered to rat pups on P7–P14 and the aberrant connections of climbing fibres on the distal territory of the Purkinje cells was observed, as was evident in the comparison of the number of VGlut2 immunoreactive marks in the distal field and the percentage of the extension of CFs into the molecular layer in the EXP group (see Fig. 2). No visible motor alterations were observed in the rats in their performance in the plus maze or in the operant chamber.

Cerebellum has been proposed to be involved in the detection of perceptual sequences in both human beings and experimental animals. The role of cerebellum has been particularly emphasized in detecting the absence of an expected stimulus (Ivry 2000) and patients with cerebellar damage fail to recognize spatial sequences (Molinari et al. 1997). Additionally, cerebellar participation in acquisition of new sequences has been observed, whereas the cerebellar dentate nucleus appears to be involved in the execution of previously learned visuomotor sequences but not in the learning of new sequences (Hikosaka et al. 1998, 1999), and lesions of the dentate nucleus disrupt the learning of egocentric-based spatial sequences (Gaytan-Tocaven and Olvera-Cortes 2004). In the present work, the rats were trained in one egocentric-based spatial sequence under forced conditions. Thus, during the first 7 days of training, detection of the

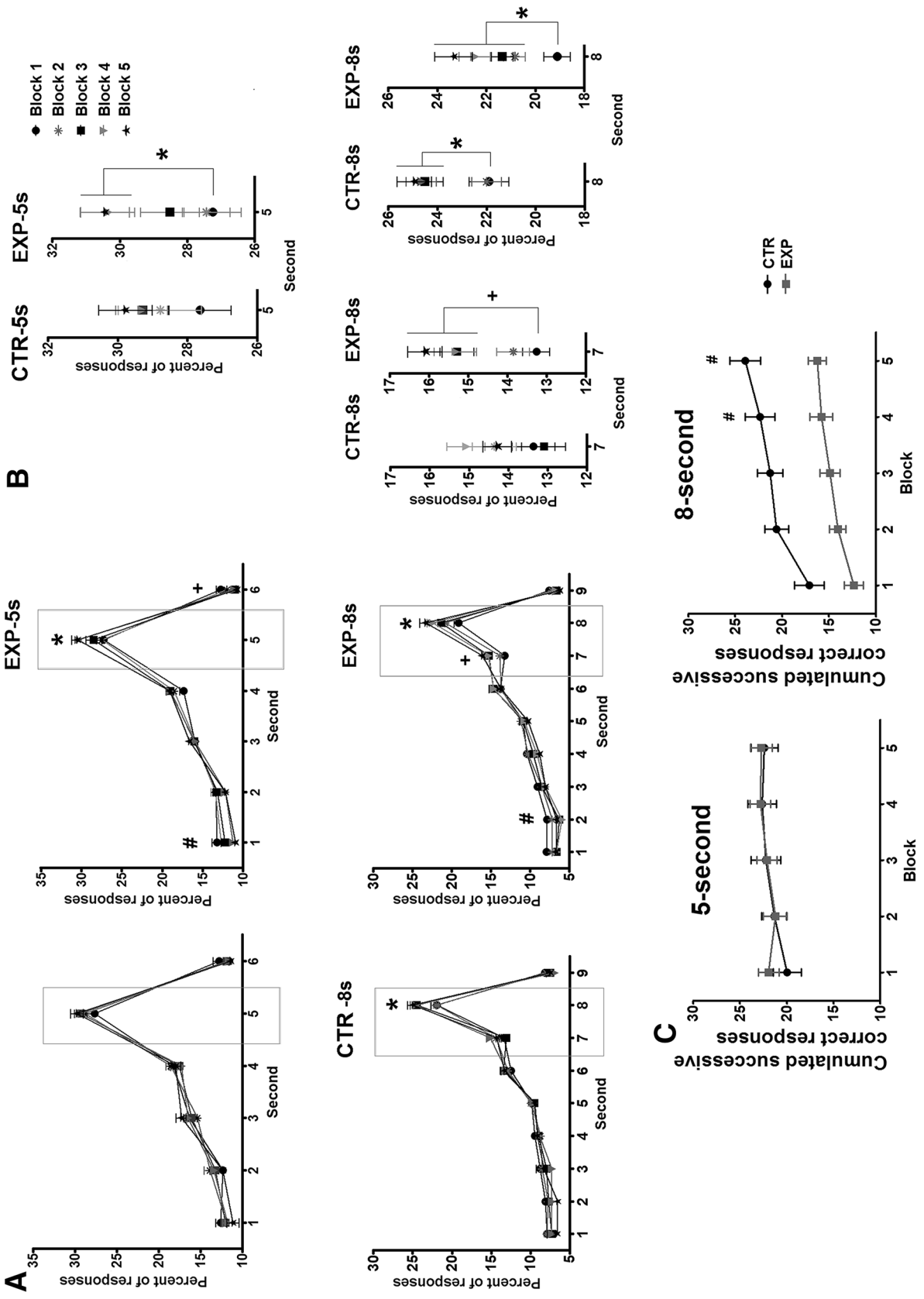
sequence of baited arms was not required for the rats. After the first 7 days, the training was free, and the four arms were opened such that the rats had to choose the baited arm among the four arms of the maze. Unlike what we expected, no differences in the number of errors were observed between groups, but in the SS, the EXP group was not affected by the new information added when the four arms of the maze were opened and remained acting as though no new options existed in the choice of arms, whereas the CTR group had a lower SS on the first days of free training. Two points can be highlighted from these results. First, the aberrant connections of CFs and PCs do not impair the learning of a sequence under forced conditions in which no detection of the sequence is required. Second, the EXP group continued executing the sequence at the same level when free choice was permitted. Thus, the aberrant connection of CF–PC did not affect the execution of a sequence learned under forced condition (under which detection of the sequence was facilitated). A possible interpretation of these results could be related to cerebellar participation in exploration. Cerebellum-damaged rats display restricted patterns of exploration in which they do not explore all of the areas in a field but are restricted to smaller areas (Molinari et al. 1997) and are impaired in representing new environments (Mandolesi et al. 2003). In this sense, in the exploration of the maze during the habituation session the experimental rats visited all the four arms of the maze. This implies that the rats were able to explore the environment in totality, in oppossition to the previous argument. However, for the other side, cerebellar mutant mice present a reduction in spontaneous alternation (Lalonde et al. 1988), and mutant mice with reduced PC number present repetitive behaviour and behavioural inflexibility in an operant lever-pressing task (Dickson et al. 2010; Martin et al. 2010). In accordance with this, both perseverant behavior and inflexibility, once the learned sequence was acquired by the experimental animals, could account for the undisturbed performance of this group in the egocentric sequential task under free choice. Although apparently better in performance, it is important to note that an alteration in adaptive behavioural responses is not beneficial for the individual. In fact, in the detection and adaptation of the behaviour after a change in the environment, the behavioural flexibility is a very important adaptive response, which could be overridden in the experimental animals which continued acting in the maze as if no additional information existed in the environment. This could give place to a higher number of enlaced responses in the plus maze, but not necessarily to the adaptation of new circumstances.

The aberrant connections of climbing fibres on Purkinje cells caused a subtle impairment in the timing of an operant response in the supra-second range, according to the hypothesis that motor and cognitive processes result similarly affected with an alteration of the cerebellar circuit. The experimental animals were able to significantly increase the number of responses in the rewarded second, both in the 5- and 8-s FI. However, in contrast to the control group, the increase was gradual in the 5-s FI, and the number of responses on the adjacent second (second 7) was also increased in the 8-s FI in the EXP group. Moreover, the biggest effect was observed in the number of successive correct responses in the 8-s FI, in which the EXP group performed significantly below the execution of the CTR group. These results are in line with the role of the cerebellum in motor learning as a device for fine coordination of motor skills (Thach et al. 1992) and with the observed participation of the PF-PC synaptic changes, in the optimization of the motor response after the association is made, but not in the establishment of stimulus–response associations (Burguiere et al. 2010). In the present work, we consider the expression of successive correct responses as the maximal optimization of the performance since the rats emitted successive responses in the exact rewarded second without any other response through the interval.

Cerebellum, striatum and cerebral cortex have been included in models of timing mechanisms, acting together for coding interval timing (Ivry and Richardson 2002), and a dual model has been proposed in which the cerebellum participates in sub-second temporal processing whereas the basal ganglia participates in supra-second ranges (Ivry 1996).

In this sense, the MK-801 ip administration could be related to disturbances in other systems involved in timing and sequential learning. However, NMDA receptors in the striatum are functional until P14 (Colwell et al. 1998), and the postnatal administration of the same antagonist caused a reduced number of cortical–striatal synapses only on P20 but not earlier (Butler et al. 1999). Moreover, the prefrontal cortex showed moderated changes in basal dendritic length in pyramidal neurons of layer III after the administration of MK-801 from P1 to P21, but not changes in the apical dendrite or dendritic spines (Wedzony et al. 2005). Thus, the changes observed in the timing in operant responses in the present study are more likely related to cerebellar aberrant CF–PC connections, than to striatal or prefrontal damage.

The present results appoint a role of the cerebellum in the fine precision of timing in the supra-second range, which was more pronounced in the long 8-s interval. Although no inter-group differences were observed in the percent of responses in second 8, the experimental group had a more gradual increase in the responses in second 8 through the training, also their number of responses in the second 7 increased significantly implying a minor accuracy (see Fig. 4b); the previous, joined to the minor


A
EXP-5s
CTR -8s
EXP-8s
CTR-8s
Percent of responses
Second
B
CTR-5s
EXP-5s
Block 1
Block 2
Block 3
Block 4
Block 5
C
5-second
8-second
CTR
EXP
Cumulated successive correct responses
Block

◂**Fig. 4** **a** Intra-group comparison of the percentage of responses performed each second for five blocks of 4 days. Percent of lever responses during the 5-s FI schedule of reinforcement for the CTR (CTR-5 s) and EXP (EXP-5 s) groups. Mean ± SE. EXP group: *, second 5, b1 vs. b4 and b5; +, second 6, b1 vs. b5; #, second 1, b1 vs. b4 and b5. $p < 0.05$. *Middle* percent of lever responses during the 8-s FI schedule of reinforcement for the CTR (CTR-8 s) and EXP (EXP-8 s) groups. Mean ± SE. CTR group: *, second 8, b1 vs. b3, b4 and b5. EXP group: *, second 8, b1 vs. b2, b3, b4 and b5; +, second 7, b1 vs. b3, b4 and b5; #, second 2, b1 vs. b4. $P < 0.05$. **b** Amplification of percentage of responses from the CTR and EXP groups emitted in second 5 (signalled by a *grey rectangle*) in the FI of 5 s; and in seconds 7 and 8 (signalled by a *grey rectangle*) in the FI of 8 s; significant differences between the block 1 and the other blocks as was described in the section A. **c** Number of cumulated sequential correct responses in the 5- and 8-s FI for the CTR and EXP groups. Mean ± SE. # b1 vs. b4 and b5. Significant group effect was observed in the 8-s FI with higher values for the CTR group

capacity of the rats to emit sequences of responses each 8 s (without any other response among the interval) indicate a disruptive effect of the aberrant connection between climbing fibres and PC. In accordance with the present results, it was observed that patients with focal lateral cerebellar damage showed increased variability in an interval production task in the supra-second range (8, 12 and 21 s), but did not show inability to execute the task (Malapani et al. 1998). In a work assessing the role of cerebellum in timing, the lesion of the IN did not alter the discrimination between tones of 2 and 8 s of duration (Callu et al. 2009). In the present results we observe discrepancies in the timing for the 8-s period, possibly because the production of intervals and not the discrimination between short- and long-duration stimulus was evaluated. Thus, the accuracy in interval production is apparently disrupted by cerebellar aberrant connection. Interestingly, Callu et al. also reported a disruption in the temporal sensibility in the lesioned group, evident in the response to the long period (8 s), which was overridden with the training (Callu et al. 2009). However, in an evaluation of patients with cerebellar lesions, Gooch et al. (2010) observed deficiencies in the production of durations of 2, 4, 6, 8, 10 and 12 s, which were more evident in shorter (2 s) than in longer (12 s) durations. The authors do not compare the net duration produced by the patients, but the proportional error, defined as (production − target duration)/target duration; thus, a minor proportional error, for long durations has yet a net error of several seconds such that the cerebellar lesioned patients are widely affected in the production of supra-second durations both for short and long durations. Based on this, the reason for the absence of differences in the 5-s FI remains elusive, and a proposition about the causes will result only in speculation. However, the present results add evidence of the participation of the cerebellum in the timing in the supra-second range.

In conclusion, the aberrant connections between CFs and PCs disrupted the fine-tuning of operant-timed responses in the supra-second range, and altered the sequential learning of egocentric information, in the adult rat.

Acknowledgements This work was supported by the Fondo para la Investigación en Salud, of the Instituto Mexicano del Seguro Social, Grant number: FIS/IMSS/PROT/G10/850. The authors thank Dr. Esperanza Melendez from the Laboratorio de Ecofisiología Animal, Universidad Michoacana de San Nicolás de Hidalgo for providing training in the technique of immunohistochemistry for labelling VGlut2. The authors thank Dr. Sergio Meneses from the Instituto de Neurociencias, Universidad de Guadalajara, for the support in the realization of the present work.

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