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Is the parvocellular red nucleus involved in cerebellar motor learning?

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Abstract

The anatomical connections of the parvocellular red nucleus (RNp) have led to the suggestion that it might participate along with the cerebellum in modifying old and developing new programs for the control of complex, compound, coordinated movements of multiple body parts. RNp projects to and excites the inferior olivary nuclear neurons, which send climbing fibers to excite neurons in contralateral cerebellar cortex and nuclei. RNp receives excitatory inputs from ipsilateral cerebral cortex (onto distal dendrites) and from contralateral cerebellar nuclei (onto proximal dendrites). We here further develop a hypothesis as to mechanism, and offer preliminary evidence from RNp inactivation studies in awake, trained macaques during modification of their gaze-reach calibration while wearing wedge prism spectacles.

Keywords

parvocellular red nucleus; gaze-reach learning; macaque

INTRODUCTION

A number of studies have focused on the essential role of the cerebellum in the trial-and-error modification of the gaze-reach calibration using wedge prisms that dissociate visual and motor coordinates. These studies have shown that in macaques cerebellar neuron activity is associated with prism adaptation and learning [8,18].

Further, cerebellar inactivation impairs in both macaques [2] and humans [15,16] both prism adaptation and learning. The question still remains as to how the nervous system makes use of a performance error, since knowledge of the error is delayed until after the task has been performed. This error information must somehow be stored and then used to adjust the performance appropriately on subsequent trials as they are planned and performed.

Here, we refine and attempt to test a mechanism, first suggested by Kennedy [11,12] that might involve the parvocellular red nucleus, (RNp). Our model is further influenced by the pioneering studies of Evarts and Tanji on the relative roles of “presetting” and “triggering” inputs to cerebral motor corticospinal neurons [20,4]. The RNp receives projections from prefrontal and premotor areas, as well as deep cerebellar nuclei [9]. These cerebral cortical areas are known to be involved in visually guided gaze-reach movements [13,14]. The RNp in turn projects to the inferior olive, which gives rise to the climbing fibers that synapse onto Purkinje cells in the cerebellar cortex [1]. Evidence suggests that memories for gaze-reach

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calibrations are stored in cerebellar cortex [2,10,6,15,16,17]. Kurata and Hoshi suggested that after an incorrect gaze-reach trial, an error signal is coded as maintained discharge (working memory) in prefrontal and premotor cerebral cortex. The cerebral cortical projection falls upon distal dendrites of RNp cells, while the cerebellar nuclear input falls upon proximal dendrites. Thus, error-coded, maintained cerebral cortical discharge falling on distal dendrites could theoretically "pre-set" the RNp cells to fire [20]. On the next subsequent trial, the cerebellar nuclear input falling upon proximal dendrites would trigger discharge from those preset cells [4]. These preset and triggered RNp cells would project to the inferior olive, and would in turn trigger a climbing fiber complex spike. The error-coded action-contingent complex spike would arrive in the cerebellum just as the next subsequent trial was being planned and initiated. Such a mechanism would allow the error signal to "wait" until it is needed and can be used for error correction. We predicted that silencing the output from the RNp would therefore prevent application of the error memory in cerebral cortex to error-correcting programs in the cerebellum. Thus, RNp inactivation would preclude adaptation to and learning of a novel prism because the error signal necessary to train would not reach the cerebellum.

EXPERIMENTS AND RESULTS

Three Rhesus monkeys (*Macaca mulatta*) performed a visually guided reach task with the right hand, touching a randomly located target on a touch-sensitive video screen (distance=21 cm). After they learned the baseline task, they were trained to look at the target through Fresnel prisms that bent the light path to the right or to the left. To see the target, they had to shift their gaze along the axis of the bent light path. On initial trials, they reached in the direction of the deviated gaze, and thus missed the target to the right or to the left. They could see the error of the reach and over many trials learned to reach off-gaze proportionate to the diopter of the prisms in order to hit the target. Upon removal of the prisms, they now foveated the target, but the new gaze-reach calibration was retained, and they missed the target by the same distance in the opposite direction from when the prisms were first donned. Over repeated trials, they gradually readapted the gaze-reach calibration to reach to and touch the target. After many repetitions of no-prism, known-prism trials, they retained both gaze-reach calibrations, and could hit the target on the first trial, no-prism or known-prism. But when introduced to a different prism of novel diopter, they underwent the trial-and-error adaptive process all over again. Injections of the GABA agonist muscimol into the parvocellular red nucleus had no effect on the baseline orthogonal gaze-reach calibration, slightly degraded the learned known-prism gaze-calibration, and prevented new gaze-reach calibration to prisms of novel diopter.

After injections, trials were performed in blocks of either "no-prism" or a prism of a certain diopter (mounted in a frame between the eyes and the screen) specifying degrees of deviation of the light path. Monkeys were trained until there was no statistical difference between the first trials of either no-prism or known-prism reaching. Then surgery was performed, consisting of cutting a hole in the skull and mounting a Lucite chamber to an acrylic skull cap for single cell recording and injection of muscimol, stereotaxically positioned approximately 8 mm anterior of the interaural line and extending 2 mm to either side of the midline. After locating the RNp by recording, 8.8 mM muscimol was injected for temporary inactivation of this portion of the nucleus. Histology was performed to verify injection locations (see [7] for further technical details).

The first monkey (Ch, female) was trained on no-prism and known-prism of R 20 diopter that bent the light path and deviated gaze 11.4 degrees to the right of the target (Fig. 1). In Fig. 1A, the first 200 trials showed no-prism gaze reach with touch centered on the target. At trial 200, the known R 20 diopter prisms were introduced. After the prolonged known-prism

training, the touch was centered almost directly on-target, with a slight tendency still to deviate to the right more on first trials than the last. Upon removing the prisms, there was a slight no-prism adaptive aftereffect to the left (down) on trial 400. In Fig. 1B, the first 270 trials (no-prism, known-prism) were similar to those in Fig. 1A. However, on trial 270, muscimol was injected into the RNp. Subsequently, there was slightly more scatter in the touches around the target, but still centering close enough to the target to obtain reward, and again with a slight tendency for aftereffect on the first no-prism trials. At trial 380, introduction of a novel L 20 diopter left-shifting prism showed deviation to the left (down) in the first trials, followed by adaptive centering of the touch on target. Upon removal of the novel L 20 diopter prisms, the touches were more displaced to the right (up), were more scattered, and showed no tendency to adapt toward the target location. We are uncertain why this was the case in this experiment, since subsequent experiments in 2 more monkeys suggested that adaptation and learning of gaze-reach deviation ipsilateral to the side of the injected red nucleus was normal. We suspected possible spread to areas outside RNp, such as RNm. Whatever the explanation, on the day subsequent to the muscimol injection (Fig. 1 C), the no-prism and the known-R 20 diopter prism behavior was similar to that before the injection as in Fig. 1 A. Furthermore, introduction of the novel L 20 diopter prisms at trial 420 not only showed adaptation, but also (upon removal of the prisms) a robust aftereffect with an initial right deviation followed by centering on the target. In sum, muscimol inactivation of RNp on one side had no effect on no-prism behavior, and only moderately impaired (i.e., scattered) known-prism behavior for deviation of gaze-reach to the side opposite the inactivation. Gaze-reach calibration for deviation ipsilateral to the side of injection was spared.

In a second monkey (Fa, female), a total of 9 injections of muscimol were made into RNp, 6 on the left and 3 on the right. In Fig. 2A [On 7 Mar 06], the animal first performed no-prism then R 20 diopter known-prism trials, and the behavior appeared similar to that of the first monkey in Fig. 1. Muscimol was then injected (arrow) into the Left RNp. No-prism trials were similar to those prior to muscimol injection, while R 20 diopter known-prism trials were again more scattered about the target. This was even more pronounced on a second set of no-prism, known-prism trials, although the known prism trials still had a tendency to group around the target. Fig. 2B shows a set each no-prism and R 20 diopter known-prism trials. Then a second injection (arrow) of muscimol was delivered into the Left RNp, again with little affect on no-prism and scattering of known prism trials. Fig. 2C shows performance one day after a third injection of muscimol into the *Right* RNp [1 Jun 06]. No-prism and R 30 diopter known-prism trials were little affected. Subsequent exposure to known L 30 diopter (17.1 degrees) prisms showed some initial adaptation (progressing from left deviation to target centering), but upon removal of L 30 diopter known-prisms, there was little or no aftereffect (i.e., little or no progression from right deviation to target centering). In sum, for this animal, as for the first, muscimol injection into RNp spared no-prism calibration and slightly affected (scattered) known-prism calibration for gaze-reach deviation to the side opposite the injection. Gaze-reach calibration for deviation ipsilateral to the side of injection (not shown) was again spared.

Nevertheless, the question remained as to how RNp inactivation would affect adaptation to and learning of prisms of *novel* diopter for gaze-reach shifts to the *opposite* side. To address this question and third animal (Bj, male) was studied. For this animal, the effects of RNp inactivation on no-prism gaze-reach calibration (none) and on contralateral known-prism calibration (slight) were similar to those described above. Fig. 3 [4 Dec 07] shows the results one month following the 15th muscimol inactivation of Left RNpc. Localization was confirmed by MRI of the manganese-labeled muscimol injection immediately after the injection. Further, subsequent histology showed neuron loss and gliosis in Left RNp. The figure illustrates the loss of ability to adapt to opposite shifting prisms of *novel* diopter. The

first set of trials was with R 5 diopter (2.85 degrees) novel prisms. These were set in goggles mounted closer to the eyes in this animal, accounting for the greater deviation of gaze-reach for the smaller diopter prisms than for the prior two animals. The distribution of touches was bimodal, some far to the right (up) of target proportionate to the calculated displacement, with some closer to the target. This we attribute to the tendency for voluntary correction ("guessing") of the observed reach-touch error on a prior trial, in the absence of the automatic gaze-reach coordination leaning process. We have seen this tendency in human studies of voluntary compensation for attempted automatic prism adaptation [16]. The second set of trials was no-prism, and gaze-reach-touch was centered on the target. The third set of trials was with novel L 5 diopter *left-deviating* prisms. Adaptation was robust with left deviation of the first touches and then centering on subsequent trials, and an initial no-prism right-deviated aftereffect with subsequent target centering. A repeat then of the novel R 5 diopter *right-shifting* prisms again showed blocked adaptation and aftereffect in the contralateral direction of gaze-reach-touch. In sum, muscimol inactivation of the RNp blocked adaptation to contralateral shift. This is consistent with the proposed model of the role of RNp in previous trial error storage for subsequent trial error correction and motor learning.

DISCUSSION

This study showed: 1) the "no prism" and same-side gaze-reach calibrations remained unchanged by ipsilateral RNp inactivation; 2) there was slight impairment of a prior-learned (known) contralateral gaze-reach prism calibration to the side opposite the injection; 3) there was loss of the ability to adapt to and presumably to learn a new calibration for gaze-reach in the opposite direction for a novel diopter; and 4) there was no negative aftereffect upon removal of the novel prisms.

Why did RNp inactivation block adaptation and learning of novel gaze-reach calibrations in the contralateral direction? The RNp receives input from the ipsilateral premotor and prefrontal areas of the cerebral cortex. These areas are known, when electrically stimulated, to drive gaze to the contralateral side. Also, these areas carry maintained discharge for periods following a prism gaze-reach error, *if* that error is to be used to correct performance in subsequent trials [13,14]. Further, these areas of cerebral cortex project to and excite the distal dendrites of RNp neurons. These same neurons receive excitatory inputs from the contralateral dentate nucleus of the lateral cerebellum. The RNp neurons project to the ipsilateral inferior olive, whose projection crosses the midline to excite the contralateral dentate nucleus and neurons of the cerebellar cortex including Purkinje cells. The lateral cerebellar cortex has been shown to be critically involved in learning and storing ipsilateral gaze-reach calibration in prism adaptation [2,15]. Our RNp model proposes that gaze-reach error information is stored as a maintained discharge signal in contralateral cerebral cortex. This signal would project to and put an excitatory bias on the distal dendrites of the RNp neurons, which would be coded for the gaze-reach error magnitude and direction of the previous trial. Thus biased, the RNp neurons are set to fire when and only when they are excited from the input of the dentate nucleus, which is a command signal (via thalamus to cerebral cortex) for initiating the *next subsequent* trial. The concatenation of the error bias signal from the previous trial and the command signal for the subsequent trial is proposed to cause those selected RNp neurons to fire and excite neurons in the ipsilateral inferior olive. These neurons in turn would excite neurons in dentate and cerebellar cortex, which, interacting with mossy fiber-parallel fiber inputs, would cause long term depression of Purkinje cell (Pc) responses to subsequent parallel fiber input. This Purkinje context-specific parallel fiber suppression would in turn lessen Pc inhibitory influences on the deep nuclei, which would tend to increase their firing rate in subsequent trials. These changes in firing, coded and compensatory for the errors in previous trials, would tend to correct the

subsequent performance. This mechanism would then account for the role of the cerebellum in controlling the adaptation and learning of the multiple calibrations in coordinated gaze-reach behaviors. Damaging the mechanism at the level of the frontal cerebral cortex, RNp, inferior olive would prevent error-driven changes in the coordinative programs stored within the cerebellum. Indeed, Kurata and Hoshi showed results similar to ours following inactivation of the ventral but not dorsal premotor cortex.

Why did RNp inactivation *not* remove the previously learned stored calibration when using *known* prisms for gaze-reach in the opposite direction? In an earlier preliminary presentation of results, from the second monkey [21], we had thought that RNp inactivation *did* remove the learned calibration of the known prisms. But upon re-analysis of the results in that animal, and comparison with the results from the two other animals, we conclude that RNp inactivation caused the learned calibration to *degrade* (accounting for the scatter compared to no-prism gaze-reach performance), but not totally erased (accounting for the tendency to center the scattered touches around the target location). This in turn would suggest that the recently learned and stored information in the calibration depends, to some extent, on continued error-driven information from the inferior olive, RNp, and cerebral cortex.

Why did RNp inactivation conspicuously *spare* calibration for the no-prism performance? This could be for a number of reasons: 1) gaze-reach behavior may, to a large extent, be hard-wired in brainstem circuits. 2) To the extent that gaze-reach behavior may have to be fine-tuned through learning, it has been practiced for a long time-the lifetime of the individual up to that point. Both explanations could contribute to its robustness despite RNp inactivation.

We are fully aware that these observations and inferences are preliminary and provisional. The behavioral task is complex: 1) the training is difficult (witness the tendency to voluntarily “guess” during the early trained even without RNp inactivation) 2) muscimol injected into the brainstem often caused the animal to not continue working (see below), and 3) (because of these factors) there were inconsistencies of results across injections and animals, and finally, because of these complexities, the protocol for each animal was somewhat different. Thus, our test of the hypothesis required the training of normal no-prism gaze-reach to and touch of target, plus training during extended exposure to known-prisms of a fixed diopter, plus exposure to novel-prisms of a different diopter, plus prism shifts in both directions, and finally, evaluation of all these behaviors to repeated injections of muscimol into the RNp. Further, the RNp is a small target in the brainstem, and is adjacent to areas which may cause behavioral deficits that would confound pursuance and interpretation of results. RNp is anterior to RNm; RNp extends about 3.0 mm and RNm about 2 mm in the AP dimension. The interstitial nucleus of Cajal (inC) is adjacent to the medio-dorsal boundary of the two, extends a little over 2 mm in the AP dimension, approximately at the junction of RNp and RNm. This gives a target window on RNp of just under 2 mm in the AP dimension and about 2 mm in the mediolateral dimension. Given the tendency of muscimol effects to spread approximately 1 mm from the center of the injection, the target is small and the chances of hitting behavior-compromising adjacent structures are high. One such complication we encountered in the third animal (injections 1–7, 10, 11) was a profound torticollis involving head, neck (and gaze) immediately after injection of the muscimol and persisting for a day or more. The face and eyes were turned to the side of the injection, the head tilted down on the opposite side. This has been observed previously in macaques to result from muscimol inactivation of the inC [3,5], and serves to identify the injection site in relation to the desired target of RNp [19]. A second complication (second animal, first and only injection) involves the probability of hitting RNm, which may have resulted in the slowing and irregularity of reaching and digit individuation. A third complication (injection 3, second animal; injections 9, 14, third animal) consisted of an

apparent lethargic euphoria (animal refused to work, yet was playful and fed voraciously) which may be attributable to the psychotropic effects of muscimol *per se*. In sum, we are aware of the shortcomings of the data as to their consistency, and the need for confirmation by other investigators and perhaps other methods. But given the number of variables involved in the experiment, we thought it best to submit our data and interpretations as they stand, in hopes that they will inspire subsequent studies in other laboratories.

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REFERENCES

1. Allen GI, Tsukahara N. Physiol. Rev. 1974; 54(4):957–1006. [PubMed: 4370744]
2. Baizer JS, Glickstein M. J. Physiol. 1974; 236(1):34P–35P.
3. Carpenter MB, Harbison JW, Peter P. J. Comp. Neurol. 2004; 140(2):131–1539. [PubMed: 4990558]
4. Evarts EV, Tanji J. J. Neurophysiol. 1976; 39(5):1069–1080. [PubMed: 824410]
5. Farshadmanesh F, Klier EM, Chang P, Wang H, Crawford JD. J. Neurophysiol. 2007; 97(3):2322–2338. [PubMed: 17229829]
6. Gilbert PFC, Thach WT. Brain Res. 1977; 128:309–328. [PubMed: 194656]
7. Goodkin HP, Thach WT. J. Neurophysiol. 2003; 89:884–895. [PubMed: 12574466]
8. Gregor B, Norris SA, Thach WT. J. Neurophysiol. 2004; 91:576–582. [PubMed: 12878717]
9. Humphrey DR, Gold R, Reed DJ. J. Comp. Neurol. 1984; 225:75–94. [PubMed: 6725640]
10. Ito M, Sakurai M, Tongroach P. J. Physiol. 1982; 324:113–134. [PubMed: 7097592]
11. Kennedy PR, Gibson AR, Houk JC. Brain Res. 1986; 364(1):124–136. [PubMed: 3947959]
12. Kennedy PR. Trends Neurosci. 1990; 13(12):474–479. Review. Erratum in: Trends Neurosci., 1991, 14(1), 13. [PubMed: 1703677]
13. Kurata K, Hoshi E. J. Neurophysiol. 1999; 81(4):1927–1938. [PubMed: 10200227]
14. Kurata K, Hoshi E. J. Neurophysiol. 2002; 88(6):3118–3132. PMID: 12466435 [PubMed - indexed for MEDLINE]. [PubMed: 12466435]
15. Martin TA, Keating JG, Goodkin HP, Bastian AJ, Thach WT. Brain. 1996a; 119:1183–1198. [PubMed: 8813282]
16. Martin TA, Keating JG, Goodkin HP, Bastian AJ, Thach WT. Brain. 1996b; 119:1199–1211. [PubMed: 8813283]
17. Medina JF, Lisberger SG. Nat. Neurosci. 2008; 11(10):1185–1192. [PubMed: 18806784]
18. Norris SA, Greger B, Hathaway EN, Thach WT. J. Neurophysiol. 2004; 92:1867–1879. [PubMed: 15128755]
19. Paxinos, G.; Huang, X-F.; Toga, AW. The Rhesus Monkey Brain in Stereotaxic Coordinates. New York: Academic Press; 2000.
20. Tanji J, Evarts EV. J. Neurophysiol. 1976; 39(5):1062–1068. [PubMed: 824409]
21. Yttri, E.; Smith, A.; Reid, E.; Thach, WT. Inactivation of parvocellular red nucleus impairs prism adaptation to and learning of contralateral gaze-reach shift; Abstr. SFN Ann. Meeting; 2006.

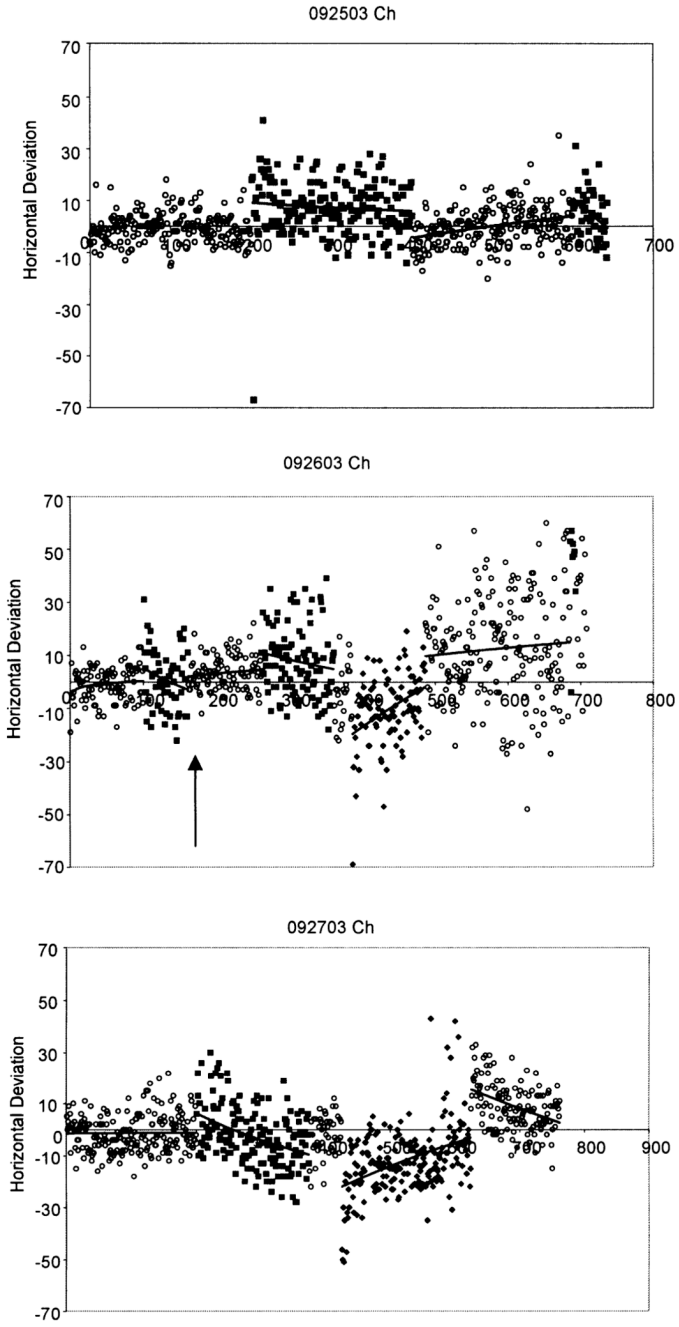

Fig. 1.

(First animal, Ch) A, one day prior to muscimol injection into L RNpc. B, day of muscimol injection. C, one day after muscimol injection. Y-axis: horizontal deviation on touch-screen in mm; right=up, left=down. X-axis: trial number. Symbols: Empty circle, no-prism; filled square, known-prism; filled diamond, novel prism. Chi-square straight line fits.

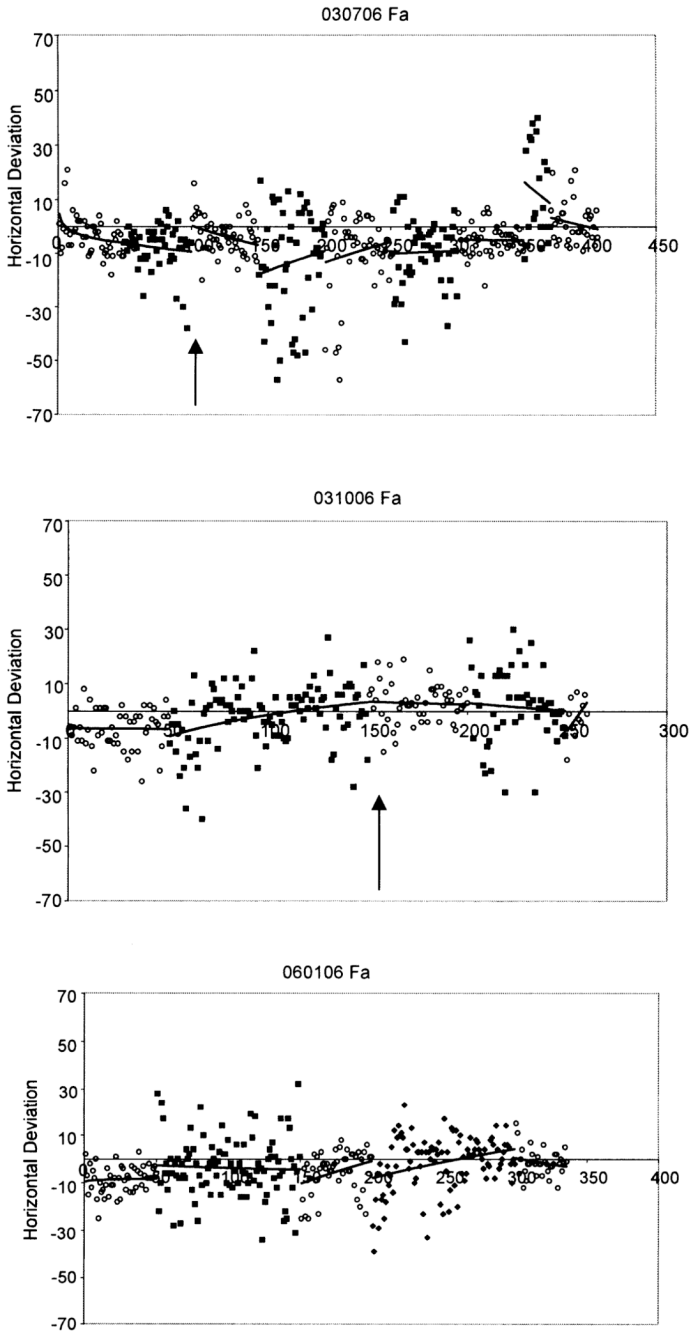

Fig. 2.

(Second animal, Fa) A, day of first injection of muscimol injection into Left RNpc. B, day of second injection of muscimol injection into Left RNpc. C, one day after fourth injection of muscimol injection into Right RNpc. Y-axis: horizontal deviation on touch-screen in mm; right=up, left=down. X-axis: trial number. Symbols: Empty circle, no-prism; filled square, known-prism; filled diamond, novel prism. Chi-square straight line fits.

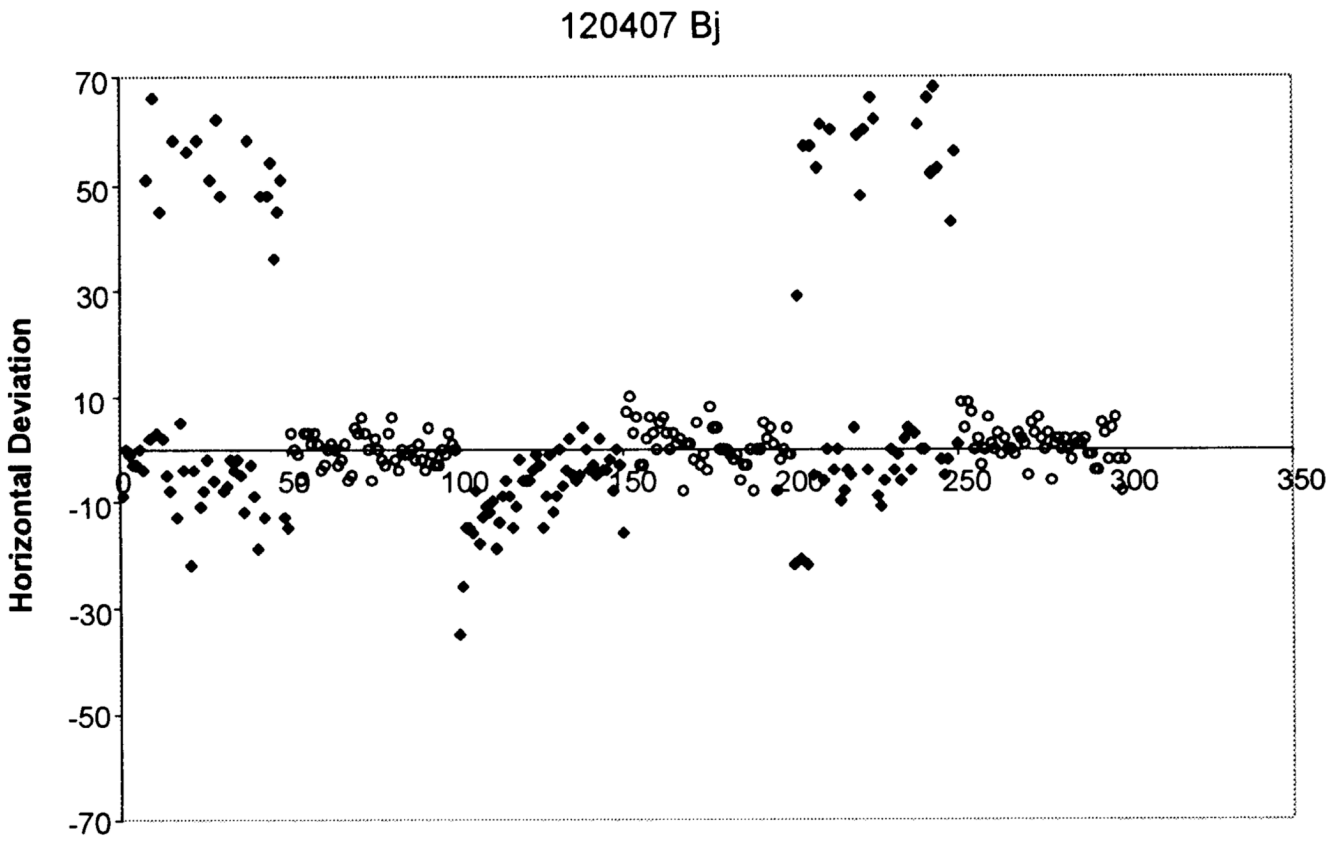

Fig. 3.

(Third animal, Bj) One month after 15th injection of muscimol into L RNpc (with permanent damage). Y-axis: horizontal deviation on touch-screen in mm; right=up, left=down. X-axis: trial number. Symbols: Empty circle, no-prism; filled diamond, novel prism.