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EFFECT OF INACTIVATION AND DISINHIBITION OF THE OCULOMOTOR VERMIS ON SACCADIC ADAPTATION

Yoshiko Kojima, Robijanto Soetedjo, and Albert F. Fuchs

Department of Physiology and Biophysics and Washington National Primate Research Center, University of Washington, Seattle, Washington 98195-7330, USA

Abstract

The ability to adapt a variety of motor acts to compensate for persistent natural or artificially induced errors in movement accuracy requires the cerebellum. For adaptation of the rapid shifts in the direction of gaze called saccades, the oculomotor vermis (OMV) of the cerebellum must be intact. We disrupted the neural circuitry of the OMV by manipulating gamma aminobutyric acid (GABA), the transmitter used by many neurons in the vermis. We injected either muscimol, an agonist of GABA, to inactivate the OMV or bicuculline, an antagonist, to block GABA inhibition. Our previous study showed that muscimol injections cause ipsiversive saccades to fall short of their targets, whereas bicuculline injections cause most ipsiversive saccades to overshoot. Once these dysmetrias had stabilized, we tested the monkey's ability to adapt saccade size to intra-saccadic target steps that produced a consistent saccade under-shoot (amplitude increase adaptation required) or overshoot (amplitude decrease adaptation required). Injections of muscimol abolished the amplitude increase adaptation of ipsiversive saccades, but had either no effect, or occasionally facilitated, amplitude decrease adaptation. In contrast, injections of bicuculline impaired amplitude decrease adaptation and usually facilitated amplitude increase adaptation. Neither drug produced consistent effects on the adaptation of contraversive saccades. Taken together, these data suggest that OMV activity is necessary for amplitude increase adaptation, whereas amplitude decrease adaptation may involve the inhibitory circuits within the OMV.

Keywords

monkey; cerebellum; saccade adaptation; oculomotor vermis; muscimol; bicuculline; GABA

INTRODUCTION

In natural or behavioral circumstances that cause a persistent inaccuracy of saccadic eye movements, the brain gradually adjusts their amplitudes so they again land on target (see Hopp and Fuchs, 2004, Iwamoto and Kaku, 2010 for reviews). This motor learning or adaptation can decrease the amplitude of overshooting saccades or increase the amplitude of undershooting saccades.

Contact information: Yoshiko Kojima, 1959 NE Pacific St., HSB I421 Washington National Primate Research Center, Box 357330, University of Washington, Seattle, WA 98195-7330. ykojima@u.washington.edu Phone: 1-206-543-2490. Fax: 1-206-685-0305.

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An intact midline cerebellum appears to be necessary for amplitude adaptation of saccades. Large excisions of the oculomotor vermis (OMV) impair the adaptation to decrease saccade amplitudes shortly after the lesion (~20 days; Takagi et al., 1998). In another study involving large lesions of the OMV, monkeys could not adapt to increase saccade amplitudes when tested about 80 days after the surgery (Barash et al., 1999). Therefore, these two studies suggest that the OMV is crucial for both the amplitude decrease and increase adaptation of saccades. However, the designs of both studies make it difficult to evaluate their conclusions regarding the role of the cerebellum in saccade motor learning because they examined saccades more than a month after the lesions. Therefore, their data did not document acute deficits. The long-term deficits could have been masked by recovery mechanisms.

In this study, instead of using large excisions, we examined both amplitude increase and decrease adaptation after local injections of either a GABA_A agonist, muscimol or an antagonist, bicuculline. These pharmacological manipulations allowed us to examine the acute effects on adaptation shortly after the injections. Furthermore, we thought that the use of bicuculline might elucidate the role of GABAergic inhibition in saccade adaptation. Because the effects of the injections were reversible within a day, we also could perform multiple experiments on the same animal, especially control experiments shortly after each injection.

GABA is the major inhibitory neurotransmitter in the OMV, and many of its neurons, including its sole output neuron, the Purkinje (P-) cell, have GABA_A receptors (Laurie et al., 1992). GABAergic cells inhibit P-cells either directly or via other cells, e.g., granule cells, that synapse with them. Therefore, muscimol effectively decreases the OMV output of the injection area. On the other hand, bicuculline disinhibits the OMV. If muscimol impairs either amplitude increase or decrease adaptation, we could infer that the OMV must be intact to induce that adaptation. Similarly, if bicuculline impairs only one type of adaptation, the OMV inhibitory network must be functional to induce that adaptation.

Our previous study (Kojima et al., 2010b) showed that muscimol injections cause ipsiversive saccades to fall short, whereas bicuculline injections cause them to have an amplitude dependent dysmetria. In our current study, we tested the ability of adaptation to increase and decrease saccade amplitude from those initially dysmetric states. The two drugs affected the adaptation of ipsiversive saccades in opposite ways. Muscimol abolished the adaptation to increase amplitude but had no effect, or occasionally facilitated, the adaptation to decrease amplitude. In contrast, bicuculline facilitated the adaptation to increase saccade amplitude but impaired the adaptation to decrease it.

RESULTS

We tested the consequences of muscimol or bicuculline injections on saccade adaptation in a total of 20 experiments. For each drug, 5 experiments evaluated the adaptation to increase amplitude and 5 the adaptation to decrease amplitude. The details of those experiments, including the participating monkey, the amount of the drug injected, the estimated side of the injection and the target step used during adaptation are presented in Table 1 as injections #1 to 20.

MUSCIMOL INJECTIONS AND ADAPTATION TO INCREASE AMPLITUDE

Muscimol injections blocked the adaptation to increase amplitude to forward target steps. The amplitudes of ipsiversive saccades during 510 sequential trials in a forward adaptation paradigm of a representative injection experiment (#3) are illustrated in Fig. 1A. As we demonstrated in an earlier study on the same monkeys (Kojima et al., 2010b), muscimol

injections induce an ipsiversive hypometria (Kojima et al., 2010b). In this experiment, that hypometria could be demonstrated by the mean amplitude of the first 50 saccades of adaptation, which was only $7.26 \pm 0.45^\circ$ to a 15° target step. At the end of 510 trials of the forward adaptation paradigm, the mean amplitude had not changed (last 50 saccades: $7.44 \pm 0.49^\circ$, $p > 0.05$).

Later, after the hypometria produced by the injection had dissipated, we confirmed that the monkey could indeed still adapt saccades to forward steps in two control experiments. We used target steps of 8° to elicit saccades with amplitudes comparable to the hypometric saccades produced by the injection. In the illustrated control experiment (Fig. 1B), the forward adaptation paradigm indeed produced a clear increase in amplitude. In this control adaptation that had NOT been preceded by a muscimol injection, the mean amplitude of the last 50 saccades after 510 adaptation trials (the same number as in the injection experiment) was significantly greater than that of the first 50 ($p < 0.05$).

Fig. 1C summarizes the change in the amplitude of ipsiversive saccades caused by forward adaptation after all 5 muscimol injections (filled bars) and for the controls performed after injections #3, 4 and 5 (open bars). After each of the 5 injections, forward adaptation never produced a significant *increase* in amplitude (positive Δ Amplitude). Saccade amplitude showed either *no significant change* (#2, 3 and 4) or a *significant decrease* (#1 and 5). Note that a decrease could not be attributed to the gradual effects of muscimol directly on saccade amplitude, because we had waited for the hypometria to stabilize before testing for adaptation. In contrast, behavioral forward control adaptations after injections #3, 4 and 5 all produced significant amplitude *increases*. After muscimol injections, contraversive saccades also showed a lack of amplitude increase adaptation (Fig. 1D). Thus, adaptation to increase amplitude was abolished after all 5 muscimol injections.

MUSCIMOL INJECTIONS AND ADAPTATION TO DECREASE AMPLITUDE

After muscimol injections, ipsiversive saccades still exhibited adaptation to decrease saccade amplitude. In the exemplar experiment illustrated in Fig. 2A, the injection caused an initial decrease in the saccade evoked by a 10° target step so adaptation was tested from a mean saccade amplitude of $7.53 \pm 0.60^\circ$ (trial numbers 1 to 50). From that amplitude, the paradigm to decrease amplitude caused gradual reduction of saccade size ($p < 0.05$, Fig. 2A). A significant gradual reduction in saccade amplitude also occurred in the representative control experiment (Fig. 2B). The amplitude decrease in this injection experiment (#8) and in the average of all three associated behavioral controls was not significantly different ($p > 0.05$, Fig. 2C, *open arrow*).

After all 5 injections, intra-saccadic target steps to decrease amplitude evoked decreases in ipsiversive saccade size (Fig. 2C, negative Δ Amplitude). Significant decreases also occurred in all 15 control experiments. The average amplitude decreases after each of the injections from #6 to 9 (Table 1) and the average decreases of their associated control experiments were not significantly different ($p > 0.05$, Fig. 2C). After the remaining injection (#10), the amplitude decrease was greater than the average of its controls. In summary, four muscimol injections had no effect on the amplitude decrease adaptation of ipsiversive saccades, and one produced more than twice the amount of amplitude decrease than its controls (Table 1).

For contraversive saccades, adaptation after two muscimol injections produced amplitude decreases equivalent to those produced by their associated controls (Fig. 2D, #6 and 7), and three injections produced more (#8, 9 and 10).

Thus, muscimol never blocked adaptation to decrease amplitude of either ipsiversive or contraversive saccades, and sometimes even facilitated it.

BICUCULLINE INJECTIONS AND AMPLITUDE INCREASE ADAPTATION

After bicuculline injections, adaptation to increase ipsiversive saccade amplitude was usually greater than normal. After the representative bicuculline injection shown in Fig. 3A, amplitude increase adaptation was substantial after only 517 saccades, whereas an associated behavioral control adaptation after no injection produced about half as much increase over the same number of trials (Fig. 3B).

The adaptation paradigm produced significant amplitude increases for both ipsi- and contraversive saccades, with or without a bicuculline injection (Fig. 3C and D). For ipsiversive saccades, adaptations after four injections (Fig. 3C, #11 to 14) produced amplitude increases that were at least two times larger than those produced by their associated controls. Adaptations after the fifth injection (#15) and its associated controls produced comparable amplitude increases. For adaptation of contraversive saccades, the amplitude increase after a bicuculline injection was comparable to that produced in the associated controls (Fig. 3D; Table 1). Thus, bicuculline usually facilitated the increase adaptation of ipsiversive saccades and had little effect on the increase adaptation of contraversive saccades.

BICUCULLINE INJECTIONS AND AMPLITUDE DECREASE ADAPTATION

After bicuculline injections, adaptation to decrease the amplitude of ipsiversive saccades was consistently less than normal. For the representative injection illustrated in Fig. 4A, the change in saccade amplitude was negligible. In contrast, the associated control experiments evoked about a 2° decrease in saccade amplitude (Fig. 4B).

No significant decreases in ipsiversive saccade amplitude were evoked after three of the five injections (#17, 19 and 20, Fig. 4C), and significant decreases were evoked after the remaining two (#16 and 18). In contrast, the associated control experiments evoked at least twice the amount of amplitude decrease, on average.

For contraversive saccades, on the other hand, the bicuculline had an inconsistent effect. In 4 of 5 injections and for all the controls there were significant decreases in saccade amplitude (Fig. 4D). Two of those 4 injections (#16, 17) produced larger amplitude decreases than their associated controls. The other two produced decreases that were either within the range of control amplitude decreases (#18) or smaller (#20). For the remaining experiment (#19), adaptation after the injection did not produce a significant change in saccade amplitude. In summary, bicuculline consistently impaired adaptation to decrease the amplitude of ipsiversive saccades, but had a less consistent effect on contraversive saccades.

Figure 5 summarizes the results of all our experiments on the effect of muscimol and bicuculline injections into the OMV on adaptation to increase and decrease saccade amplitude. Here, we show the number of injections after which the adaptation was either not significant (NSΔ), or was less than, the same, or greater than the control adaptations.

For ipsiversive saccades, muscimol eliminated amplitude increase adaptation (Fig. 5A top) and had either no effect on, or occasionally facilitated, amplitude decrease adaptation (Fig. 5B top). On the other hand, bicuculline almost always facilitated amplitude increase adaptation (Fig. 5A bottom) and either abolished or slowed amplitude decrease adaptation (Fig. 5B bottom). For contraversive saccades, muscimol eliminated amplitude increase adaptation (Fig. 5C top) and either facilitated or had no effect on amplitude decrease adaptation (Fig. 5D top). In contrast, bicuculline had no consistent effect on amplitude decrease adaptation (Fig. 5D bottom) and either facilitated or had no effect on amplitude increase adaptation (Fig. 5C bottom).

DISCUSSION

We used pharmacological injections to explore the role of the oculomotor vermis in the adaptation of saccades to behaviorally induced post-saccadic errors. These reversible manipulations allowed us to examine the acute effects on adaptation and to compare them with control adaptations performed soon thereafter. Our rather small injections of muscimol and bicuculline produced opposite deficits on the adaptation of *ipsiversive* saccades. Muscimol abolished amplitude increase adaptation, but had either no effect or, in some experiments, enhanced amplitude decrease adaptation. In contrast, bicuculline usually facilitated the adaptation to increase saccade amplitude and abolished or impaired the adaptation to decrease it.

Injections of either drug caused more variable and unpredictable deficits on the adaptation of *contraversive* saccades. Our previous study also showed that both drugs caused inconsistent dysmetrias of contraversive targeting saccades (Kojima et al., 2010b). The unreliable effects on contraversive saccades may have been due to some, uneven spread of the injected agent across the midline. Because we delayed 40 min before testing the effect of muscimol, it might have had time to diffuse to the contralateral side, whereas bicuculline, which was tested immediately after the injection, might not have. Our finding that the effects on the adaptation of ipsiversive and contraversive saccades tended to be more similar after muscimol than bicuculline injections (Table 1, Fig. 5) would be consistent with our diffusion suggestion. Because of the variable effects on contraversive saccades, we will consider only processes that could be involved in the adaptation of ipsiversive saccades.

Previous studies reported that large aspirations of the monkey vermis also impair adaptation to increase and decrease saccade amplitude (Barash et al., 1999; Takagi et al., 1998). Similarly, after pathological lesions of the human OMV produced persistent overshoots, the adaptation required to decrease saccade size was impaired (Straube et al., 2001; Waespe and Baumgartner, 1992; Waespe and Müller-Meisser, 1996). In contrast to those studies, our small muscimol injections presumably inactivated only part of the OMV, because the amount of hypometria we produced was smaller than that produced by aspirations of the OMV. However, it was impossible to document either the specific cerebellar layer that was injected or the extent of the spread of the drug. The large aspirations of Takagi et al. (1998) produced hypometrias ranging from ~ 15 to ~ 50% compared to our hypometrias (Kojima et al., 2010b) of ~ 5 to 30%.

In our experiments, we injected into that area of the vermis with the strongest saccade-related activity, and we used only just the amount of drug that was required to induce a noticeable dysmetria and the appropriate corrective saccades. Nevertheless, our small muscimol injections abolished amplitude increase adaptation but had little effect on decrease adaptation. Perhaps because our monkeys showed a weaker and slower amplitude increase than decrease adaptation, amplitude increase adaptation might simply have been very sensitive to the loss of even a small portion of the OMV. In addition, the devastating affect on increase adaptation also suggests that the neuronal circuitry that underlies amplitude increase adaptation is largely concentrated in the vermis and is not distributed across other sites. In contrast, amplitude decrease adaptation remained quite robust after our partial inactivation of the OMV by muscimol. Perhaps if all of the P-cells had been affected, amplitude decrease adaptation might also have been impaired. An alternative explanation is that the major neuronal loci that subserve amplitude decrease adaptation lie outside the OMV. At this time, however, the available evidence has established only that such putative loci would lie downstream of the superior colliculus (Quessy et al., 2010).

Our muscimol and bicuculline injections clearly produced differential effects on amplitude increase and decrease adaptation. Although we cannot ascertain whether bicuculline had an equal effect on all interneurons in the injected OMV area, there is no doubt that it specifically disrupted local inhibitory transmission. That disinhibition facilitated amplitude increase adaptation and suppressed or had no effect on amplitude decrease adaptation. This suggests that inhibitory mechanisms within the OMV at least partially contribute to amplitude decrease adaptation. For amplitude increase adaptation, on the other hand, disinhibition might make it easier to increase the activity of the output of OMV. A faster increase of OMV output activity could cause a faster decrease of activity in the caudal fastigial nucleus, which, in turn, would increase burst generator activity more rapidly and increase saccade amplitude faster (e.g., as in Fig. 3).

Thus, partial inactivation and partial disinhibition of the OMV produced substantially different effects on the amplitude increase and decrease adaptation of saccades. Our data show that the OMV is crucial site for both of these amplitude adaptations, but they suggest it accomplishes them by two very different synaptic mechanisms.

EXPERIMENTAL PROCEDURE

We performed pharmacological injections into the oculomotor vermis (OMV) of two rhesus monkeys (*Macaca mulatta*, male, Monkey B: 7.4 and Monkey W: 5.0 kg), both of which also provided data for our previous study on the effects of GABA agonists and antagonists on the metrics of horizontal saccades (Kojima et al., 2010b). The methods for recording eye movements and unit activity from the OMV are described in detail in Kojima et al. 2010a. Briefly, each monkey underwent surgical procedures to implant head stabilization lugs, an eye coil and a recording chamber aimed straight down at 14.5 mm posterior to the inter-aural line on the midline. Once a monkey had been trained to track a small target spot, we recorded unit activity from the OMV with a homemade tungsten micro-electrode.

Injection of pharmacological agents

We injected either 2 μ g/ μ L muscimol (MP Biomedicals) or 5 μ g/ μ L bicuculline (Bicuculline Methochloride, MP Biomedicals). To deliver the pharmacological agents to the correct location, we placed them in a 35-gauge stainless steel tube that was insulated except at the tip so it could serve as a recording electrode. From our previous studies (Kojima et al., 2010, Soetedjo & Fuchs 2006) and those from the other lab (Catz et al., 2008, Thier et al., 2000), the oculomotor vermis is characterized by P-cells that have phasic activity associated with saccades, usually in all directions. Moreover, it is the only area of the cerebellar vermis that exhibits phasic background activity related to saccades. Therefore, in preliminary penetrations, we first demarcated the region of saccade-related phasic P-cells. Then on the day of the injection, we directed the injection cannula to this area, and lowered it until we recorded background activity related to saccades through its exposed tip. Finally, we positioned the cannula tip in the center of the phasic background activity.

After waiting 5 min to allow the tissue to stabilize, we began by injecting 100 nl of either drug. If horizontal saccades showed no dysmetria after this amount, we injected more (20 – 900 nl in different experiments) until some dysmetria was just produced (see Kojima et al., 2010b for details). The background activity decreased and increased following muscimol and bicuculline injections, respectively. The total amount of each injection in both monkeys is shown in Table 1.

Despite the small size of the injections, it is possible that some of the drug spread across the midline (Arikan et al., 2002). However, it is unlikely that injections spread to the caudal fastigial nucleus because we never observed deficits like those produced when either

muscimol (Robinson et al., 2002) or bicuculline (Sato and Noda, 1992) were injected there. Moreover, the injections did not spread to vestibular-related areas of the cerebellum because they never evoked nystagmus or drifts of the eye when the monkey was in the dark. Whereas we were confident from the recorded background activity that the tip of the cannula was indeed in the oculomotor vermis, it was impossible to ascertain which cerebellar layers or elements were affected.

Bicuculline blocks not only the GABA_A receptor but also small-conductance calcium-activated potassium (SK) channels, which help to regulate P-cell excitability (Womack and Khodakhah, 2003). However, the effect of bicuculline appears to be mostly due to a reduction of GABA_A shunting inhibition rather than blockage of SK channels (Tonkovic-Capin et al., 2001). Therefore, we think our bicuculline effects were mediated mainly through the GABA_A receptor.

In our previous study, we tested the time course of the efficacy of muscimol and bicuculline (Kojima et al., 2010b). Muscimol caused a gradual decrease in ipsiversive saccade amplitude which stabilized after ~40 min and remained so for about another hour. Therefore, after each muscimol injection, we waited at least 40 min before we tested saccade adaptation. We confirmed on-line that the effect of the drug had indeed stabilized by examining saccades to 10° target steps in 8 directions every 45° from horizontal every 10 min after the injection. During the 10 min intervals between saccade tests, the animal sat in the dark. In contrast, bicuculline produced an immediate ipsiversive saccade dysmetria, which remained stable for ~40 min. Therefore, as soon as we observed a dysmetria, we tested saccade adaptation for a period of 40 min at most.

Experimental protocols

SACCADE COLLECTION AND ADAPTATION—For every experiment, we collected at least 20 saccades to each horizontal target step of 5, 10, 15, 20 25 and 30° before the injection and after the effects of the injection had stabilized. The target moved to the various horizontal locations between $\pm 20^\circ$ pseudo randomly. During this data collection, we turned off the target for 500 ms as the saccade was launched so that the dysmetria produced by the injection did not lead to any saccade adaptation (Seeberger et al., 2002).

After collecting the pre- and post-injection data, we induced saccade amplitude increase or decrease adaptation in both the left- and rightward directions using the McLaughlin (1967) paradigm. As a monkey made a saccade toward a stepped target, we detected its occurrence with a velocity (75°/s) threshold and then jumped the target by a fixed amount during the saccade, either backward so the eye overshot or forward so it fell short. As adaptation progressed, we increased the size of the intra-saccadic adapting jump to keep the over- or undershooting error relative to the target between 2 and 3°. For amplitude decrease adaptation, we used the primary target step that had elicited the saccade that showed the greatest dysmetria after the effects of the injection had stabilized (either 10, 15 or 20°, Table 1). For amplitude increase adaptation, we used either a 10 or 15° target step, whichever was associated with the greater dysmetria. As we showed previously (Kojima et al 2010b), saccades to these step sizes are more variable after a bicuculline injection. However, we chose these amplitudes because our preliminary behavioral experiments in these monkeys showed that the greatest amplitude increase adaptation occurred for these sizes.

CONTROL EXPERIMENTS—We performed behavioral control adaptations usually on each of the 3 days following an injection to compare the amount of adaptation that was possible in the presence of either drug with that produced when it was absent. Control adaptations were collected after injections #6 to 20. Because forward adaptation was effectively abolished after all 5 of the muscimol injections, we performed behavioral

controls only after each of the last 3 (#3 [2 controls only] to 5) to confirm that the animals indeed were still able to adapt normally. We started a control adaptation from the saccade sizes that had resulted after the dysmetria produced by the drug used in the previous injection experiment had stabilized. For example, if an injection had caused 10° saccades to be reduced to a stable amplitude of 8° before adaptation, the control experiment would test the ability of normal saccades elicited by an 8° target step and exposed to intra-saccadic target steps of ~2 to 3° to adapt in the McLaughlin (1967) paradigm. Therefore, we could compare the ability to adapt with and without the drug under the same initial conditions.

Data analysis

During the experiment, a custom program running in Spike 2 (CED) calculated and displayed some features of the data on-line. These included plots of saccade amplitude vs. trial number or target amplitude from which we determined (1) if the dosage of the drug was effective, (2) when the dysmetria had stabilized, and (3) the target amplitude at which saccades were most affected. The data also were saved and analyzed off-line using custom computer programs that ran on analysis software (Spike 2, CED). This software identified each primary target step and the saccade it elicited and determined the saccade onsets and ends by an eye velocity criterion of 20°/s. These markings allowed saccade amplitude to be determined. The processed data were exported to Matlab (Mathworks) to perform statistical tests. We eliminated those saccades with vectors that differed from the target direction by $\geq 10^\circ$ or whose initial horizontal and vertical eye positions differed from those of the initial target positions by $\geq 1^\circ$ (Kojima et al., 2010a). These criteria eliminated <10% of the saccades across all experiments.

To assess how much adaptation had occurred, we compared the mean amplitudes of the first and last 50 saccades of adaptation after an injection and after each of the behavioral control adaptations. When the total number of trials differed in the injection and behavioral experiment, we took the last 50 trials in the experiment with the least data and analyzed those same trial numbers as the “last” 50 in the experiment with the most data. The average number of trials for all injection experiments was 482 (range: 135–896). After injections, if the amplitude change was either not significant ($p > 0.05$, unpaired t-test) or in the direction opposite to the adaptation, we inferred that adaptation paradigm had not produced a significant change (Fig. 5, “NSΔ” designation). If adaptation still occurred after the injection, we compared it with the average of the successive behavioral control adaptations. The average amplitude change (A_c) and SD (SD_c) of the three control adaptations ($A_{1,2,3}$) was computed as follows:

$$A_c = \frac{n_1 \cdot A_1 + n_2 \cdot A_2 + n_3 \cdot A_3}{n_1 + n_2 + n_3} \quad (1)$$

$$SD_c = \sqrt{\frac{(n_1 - 1)SD_1^2 + (n_2 - 1)SD_2^2 + (n_3 - 1)SD_3^2 + n_1 \cdot (A_1 - A_c)^2 + n_2 \cdot (A_2 - A_c)^2 + n_3 \cdot (A_3 - A_c)^2}{n_1 + n_2 + n_3 + 1}} \quad (2)$$

If the amplitude change after an injection was significantly less than that produced during a control adaptation, we inferred that adaptation after the injection was less (Fig. 5, “Less”). On the other hand, if the change in adaptation amplitude after the injection was statistically larger than that of the control adaptation, we inferred that the injection had facilitated the adaptation (Fig. 5, “More”). Finally, if the adaptation amplitude change after the injection

was statistically indistinguishable from that of a control adaptation, the injection had no effect on adaptation (Fig. 5, "Same").

These experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (1997) and exceeded the minimal requirements recommended by the Institute of Laboratory Animal Resources and the Association for Assessment and Accreditation of Laboratory Animal Care International. All the procedures were evaluated and approved by the local Animal Care and Use Committee of the University of Washington.

Research Highlights

GABA agonist and antagonist were injected into the oculomotor vermis (OMV). ► Saccade amplitude increase and decrease adaptation (UP/DOWN) were tested. ► Agonist abolished UP, but had no effect on DOWN. ► Antagonist facilitated UP and slowed DOWN. ► Results suggest OMV is necessary for UP, and DOWN may involve inhibitory circuits.

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Figure 1.

Amplitude increase adaptation after muscimol injections. **A**, Ipsiversive saccade amplitude as a function of trial number after the dysmetria due to the injection had stabilized. Squares indicate the mean amplitude of the first and last 50 saccades. Error bars throughout the figure indicate SDs. **B**, Same plot as in **A** for one of the associated control adaptations. **C**, **D**, Summary of the amplitude change produced by each amplitude increase adaptation after an injection (filled bars) and its associated control experiments (open bars) for ipsi- and contraversive saccades, respectively. § indicates the amplitude change was not significantly different from 0. Experiment #3 in **C** provided the data in **A** and **B**.

Figure 2.

Amplitude decrease adaptation after muscimol injections. **A**, Ipsiversive saccade amplitude as a function of trial number after the dysmetria due to the injection had stabilized. Squares indicate the mean amplitude of the first and last 50 saccades. Error bars throughout the figure indicate SDs. **B**, Same plot as in **A** for one of the associated control experiments. **C**, **D**, Comparison of amplitude changes produced by adaptation after an injection (filled bars) and in the associated control experiments (open bars) for ipsi- and contraversive saccades, respectively. "ns" indicates the amplitude change after injection was not significantly different from the average amplitude change of 3 controls; * indicates that it was. Experiment #8 in **C** provided the data in **A** and **B**.

Figure 3.

Amplitude increase adaptation after bicuculline injections. **A**, Ipsiversive saccade amplitude as a function of trial number after the injection. The larger variability occurs because the bicuculline induced both hypo- and hypermetric saccades at this amplitude (Kojima et al., 2010b). Squares indicate the mean amplitude of the first and last 50 saccades. Error bars throughout the figure indicate SDs. **B**, Similar plot for one of the control experiments obtained after the injection experiment illustrated in **A**. **C**, **D**, Comparison of amplitude changes produced by adaptation after an injection (filled bars) and in the associated control experiments (open bars) for ipsi- and contraversive saccades, respectively. "ns" indicates the amplitude change after the injection was not significantly different from the average

amplitude change of the 3 controls; * indicates a significant difference. Experiment #14 in **C** provided the data in **A** and **B**.

Figure 4.

Amplitude decrease adaptation after bicuculline injections. **A**, Ipsiversive saccade amplitude as a function of trial number after injection. The larger variability occurs because the bicuculline induced both hypo- and hypermetric saccades at this amplitude (Kojima et al., 2010b). Squares indicate the mean amplitude of the first and last 50 saccades. Error bars throughout the figure indicate SDs. **B**, Similar plot for one of the control experiments obtained after the injection experiment illustrated in **A**. **C**, **D**, Comparison of amplitude changes produced by adaptation after an injection (filled bars) and in the associated control experiments (open bars) for ipsi- and contraversive saccades, respectively. \$ indicates the amplitude change was not significantly different from 0. "ns" indicates the amplitude change after the injection was not significantly different from the average amplitude change of the 3 controls; * indicates a significant difference. Experiment #19 in **C** provided the data in **A** and **B**.

Figure 5.

Histogram of the number of injection experiments in which amplitude increase and decrease adaptation was not significant (NSΔ) or significantly less than, the same or more than the associated controls. **A, B**, Ipsiversive saccades. **C, D**, Contraversive saccades. **A, C**, Amplitude increase adaptation. **B, D**, Amplitude decrease adaptation. The injected drugs are indicated at the left of each row of panels.

Table 1

Summary of the conditions (first 7 columns) and the resultant saccade amplitude changes in all 20 experiments. Amplitude changes (Δ Amplitude) either were not significant ("ns"), significantly increased ($\uparrow$) or significantly decreased ($\downarrow$). Adapted saccade amplitudes after injections (I) were either more than ($I > C$), less than ($I < C$) or not significantly different (ns) from that of the combined control adaptations. "NA" indicates "not applicable" for the injection vs. control comparison because the injection produced no significant adaptation.

Injection #	Monkey	OMV side	Volume (nl)	Target step size	Drug	Adapt Paradigm	Δ Amplitude					
							Ipsi			Contra		
							Inj	Ctrl	Inj vs Ctrl	Inj	Ctrl	Inj vs Ctrl
1	B	Right	80	10°	Muscimol	increase	$\downarrow$	-	NA	$\downarrow$	-	NA
2	B	Right	120	10°	Muscimol	increase	ns	-	NA	$\downarrow$	-	NA
3 Fig. 1A	B	Left	120	10°	Muscimol	increase	ns	$\uparrow$	NA	ns	*	NA
4	W	Right	1000	15°	Muscimol	increase	ns	$\uparrow$	NA	$\downarrow$	*	NA
5	W	Right	1000	15°	Muscimol	increase	$\downarrow$	$\uparrow$	NA	$\downarrow$	*	NA
6	B	Left	200	10°	Muscimol	decrease	$\downarrow$	$\downarrow$	ns	$\downarrow$	$\downarrow$	ns
7	B	Right	620	10°	Muscimol	decrease	$\downarrow$	$\downarrow$	ns	$\downarrow$	$\downarrow$	ns
8 Fig. 2A, 6	B	Left	300	10°	Muscimol	decrease	$\downarrow$	$\downarrow$	ns	$\downarrow$	$\downarrow$	$I > C$
9	W	Right	1000	20°	Muscimol	decrease	$\downarrow$	$\downarrow$	ns	$\downarrow$	$\downarrow$	$I > C$
10	W	Right	1000	15°	Muscimol	decrease	$\downarrow$	$\downarrow$	$I > C$	$\downarrow$	$\downarrow$	$I > C$
11	B	Right	120	10°	Bicuculline	increase	$\uparrow$	$\uparrow$	$I > C$	$\uparrow$	$\uparrow$	ns
12	B	Left	140	10°	Bicuculline	increase	$\uparrow$	$\uparrow$	$I > C$	$\uparrow$	$\uparrow$	ns
13	B	Right	100	10°	Bicuculline	increase	$\uparrow$	$\uparrow$	$I > C$	$\uparrow$	$\uparrow$	$I > C$
14 Fig. 3A	W	Left	320	10°	Bicuculline	increase	$\uparrow$	$\uparrow$	$I > C$	$\uparrow$	$\uparrow$	ns
15	W	Left	360	10°	Bicuculline	increase	$\uparrow$	$\uparrow$	ns	$\uparrow$	$\uparrow$	$I > C$
16	B	Right	100	15°	Bicuculline	decrease	$\downarrow$	$\downarrow$	$I < C$	$\downarrow$	$\downarrow$	$I > C$
17	B	Left	280	15°	Bicuculline	decrease	ns	$\downarrow$	NA	$\downarrow$	$\downarrow$	$I > C$
18	B	Left	140	20°	Bicuculline	decrease	$\downarrow$	$\downarrow$	$I < C$	$\downarrow$	$\downarrow$	ns
19 Fig. 4A	W	Left	320	15°	Bicuculline	decrease	ns	$\downarrow$	NA	ns	$\downarrow$	NA
20	W	Right	320	15°	Bicuculline	decrease	ns	$\downarrow$	NA	$\downarrow$	$\downarrow$	$I < C$