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RESEARCH NOTE

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Effects of diazepam on closed- and open-loop optokinetic nystagmus (OKN) in humans

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Abstract The effects of diazepam on optokinetic nystagmus (OKN) eye movements were studied under closed-loop and open-loop conditions in healthy humans. The open-loop condition was achieved by adding the eye-movement velocity signal of OKN to the computer-generated signal controlling the moving stimulus grating. Each of four subjects received a single oral dose of 5 mg diazepam or a placebo on two separate days in a double-blind randomized fashion. OKN eye movements were measured 90 min after administration of the treatments. As compared to placebo, diazepam significantly reduced the gain of open-loop OKN, but did not modify the gain of closed-loop OKN. The results indicate that the OKN gain under the open-loop condition is a more sensitive detector of the parameter changes of the OKN system than under the closed-loop condition. Thus, open-loop OKN gain can provide an objective, quantitative measure of benzodiazepine agonist effects.

Keywords Optokinetic nystagmus · Closed-loop · Open-loop control · Diazepam · OKN gain

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Introduction

In recent years, a growing body of literature has described the effects of benzodiazepines (Bzs) on eye movements (Rothenberg and Selkoe 1981a, 1981b; Bittencourt et al. 1983; Roy-Byrne et al. 1993; Leigh and Zee 1999), since specific Bz binding sites are located in areas of the CNS known to participate in vision and oculomotor control (Möhler and Okada 1978; Speth et al. 1978). Prior eye movement studies have indicated that saccadic peak velocity and smooth pursuit tracking are impaired by administration of Bzs (Rothenberg and Selkoe 1981a, 1981b; Bittencourt et al. 1983; Roy-Byrne et al. 1993). Therefore, these findings suggested that saccadic and smooth pursuit eye movements could be utilized to assess the pharmacodynamic effects of such CNS-active drugs (Roy-Byrne et al. 1993; Hopfenbeck et al. 1995). However, the performance of both types of voluntary eye movements are susceptible to many factors, such as the attributes of the stimulus and attention (Bollen et al. 1993; Leigh and Zee 1999), which would make the measure less invariant. On the other hand, optokinetic nystagmus (OKN) eye movement is an involuntary response elicited by a large-field moving visual stimulus (Collewijn 1981). Previous Bz studies on OKN eye movements have shown that the slow phase velocity of OKN was reduced distinctly in rabbits following drug treatment (Matsunaga et al. 1983), but in goldfish it remained unchanged (Salas et al. 1992). These results in experimental animals have been contradictory, and few data are available about the effects of drugs on optokinetic responses in humans.

The regular OKN eye movement tracking the stimulus motion serves to stabilize the retinal image of the moving stimulus. This eye-movement response will reduce the image slip on the retina; thus the response output immediately interacts with the stimulus input and the OKN system forms a closed loop. Generally, previous studies in the regular OKN response have used measures of OKN such as closed-loop gain, which is a ratio of slow phase velocity of OKN to stimulus velocity (Collewijn

1981). Due to the effect of negative feedback loop, the closed-loop gain of OKN is very stable and is close to unity, which would make it difficult to sensitively measure the pharmacological influences in its pathway. For effectively detecting the changes occurring in a closed-loop system, it is necessary to break the feedback loop (open loop) (Yang et al. 2000; Tian et al. 2002). In comparison with the closed-loop OKN, the gain of open-loop OKN is much larger than 1 at low stimulus velocities, and is more sensitive to changes in the OKN neural pathway. Therefore, these factors have prompted us to examine the effects of diazepam, a classical representative of the Bzs, on OKN eye movements under closed-loop and open-loop conditions.

Material and methods

Subjects

Five medically healthy subjects aged between 20 and 29 years were recruited from graduate students and colleagues of our institute. No subject had a history of benzodiazepine or other sedative-hypnotic use or had received any medication in the past month. They were requested not to drink coffee, tea or alcohol within 12 h before the drug intake. Data were discarded on one subject who was unable to produce a continuous and regular OKN following treatments with diazepam.

Subjects gave written informed consent at the beginning of the study after all procedures were explained to them. The study was approved by the institutional human subjects review board. The experimental procedure was in accordance with the Code of Ethics of the World Medical Association reproduced in the *British Medical Journal* 18, July 1964.

Eye movement recording and optokinetic stimulation

Eye movements were recorded using an electromagnetic scleral search coil technique (Robinson 1963). An annulus of suction contact lens containing a coil (Skalar Medical, The Netherlands) was affixed to the subject's right eye after topical anesthesia was administered with oxybuprocaine hydrochloride 0.4%. Calibration was performed carefully at the beginning of each trial. Four tiny points (3 × 3 pixels of each) were sequentially presented on the screen at each position: −18° and 18° horizontally, and −12° and 12° vertically. The average value of each calibration position was displayed on a screen for monitoring.

The computer-generated stimulus consisted of black and white vertical stripes of equal width (1.8°) displayed on a computer monitor. The luminance of the white stripe was 3.53 cd/m^2 and that of the black stripe was 0.06 cd/m^2. The screen was viewed at a distance of 57 cm and subtended a visual angle of 36° × 27°. Four velocities of 1.68, 3.375, 6.75 and 13.5°/s were run under both closed-loop and open-loop conditions.

Under closed-loop condition, the horizontal velocity of the grating remained constant. Open-loop stimulus was achieved by applying external feedback from the horizontal eye movement measurement to the stimulus. The velocity of eye movement was calculated in real-time and added to the computer-generated signals controlling the movement of the stimulus grating. In this way, retinal image motion was unaffected by eye movements and a constant retinal slip velocity was maintained. Simultaneously, eye movements were displayed on another screen for on-line monitoring.

Procedure

Subjects underwent two testing days, one with 5 mg diazepam (Valium) and one with placebo (vitamin B_1), at intervals of at least 1 week. Drugs were administered orally in a double-blind, randomized fashion. That is, both the subjects and the experimenters running the trials did not know the order of diazepam and placebo administration. Moreover, the data analysis was performed by a person who had no access to information regarding the experiments. On each test day, OKN experiments under closed-loop and open-loop conditions started about 90 min after the drug treatments. Subjects remained in a dimly lit and sound-isolated room, having the head position stabilized by a chin rest. They were asked not to make any voluntary eye movements but to stare at the screen and allow involuntary responses to occur. Both leftward and rightward responses were measured under the closed-loop and open-loop conditions. The order of the two conditions was counterbalanced over subjects and drugs. Each trial lasted about 30 s. Eye movement signals were sampled at 100 Hz and stored for off-line analysis. Each experiment was limited to 30 min; at end of this time, the contact lens was removed.

Data analysis and statistical tests

A computer program was developed which first eliminated the fast phases and then calculated the average velocity of slow phases for each 30-s trial. The gain, the ratio of slow phase velocity of OKN to stimulus velocity, for each subject was calculated as the mean of leftward and rightward responses. We also calculated the relative difference in OKN gain after diazepam and placebo, defined as $(G_d–G_p)/G_p$, where G_d and G_p are the OKN gains after diazepam and placebo, respectively. The paired *t*-test was used for statistical analysis to compare the OKN gain after intake of diazepam and placebo.

Results

Closed-loop OKN responses

Samples of OKN eye movement under closed-loop condition are illustrated in Fig. 1A. As shown in this figure, there is no obvious difference between the slopes of the OKN slow phases after diazepam and placebo administration.

The OKN gain under closed-loop condition after diazepam and placebo for the four subjects are presented in Table 1A. As expected, the gain of closed-loop OKN

Table 1 Closed-loop OKN gain after administration of diazepam and placebo

Subject	1.68 deg/s		3.375 deg/s		6.75 deg/s		13.5 deg/s	
	Placebo	Diazepam	Placebo	Diazepam	Placebo	Diazepam	Placebo	Diazepam
1	0.77	0.66	0.78	0.68	0.81	0.72	0.83	0.74
2	0.92	0.91	0.82	0.86	0.77	0.78	0.72	0.60
3	0.87	0.88	0.94	0.87	0.95	0.87	0.88	0.83
4	0.90	0.99	0.91	0.79	0.90	0.73	0.89	0.60

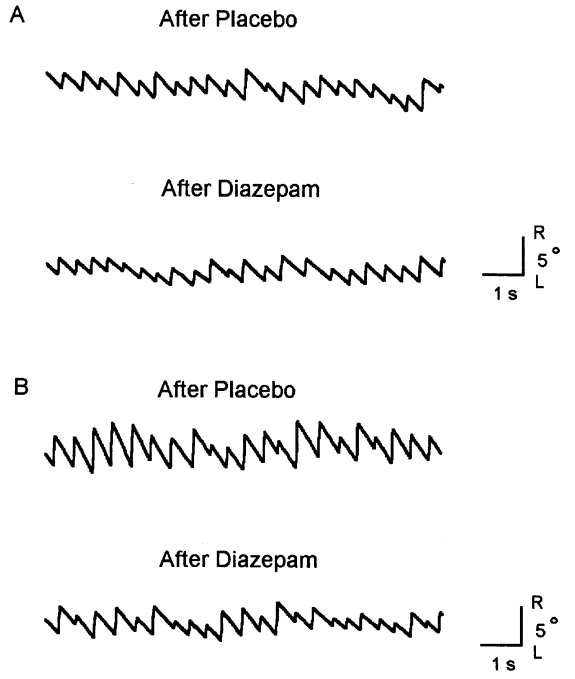

Fig. 1A, B Examples of optokinetic nystagmus (OKN) after intake of diazepam and placebo (*left*). The *upper trace* shows OKN after placebo; the *lower trace* shows OKN after diazepam. Time and eye movement calibrations are as indicated. **A** Under the closed-loop condition (6.75°/s); **B** under the open-loop condition (1.68°/s)

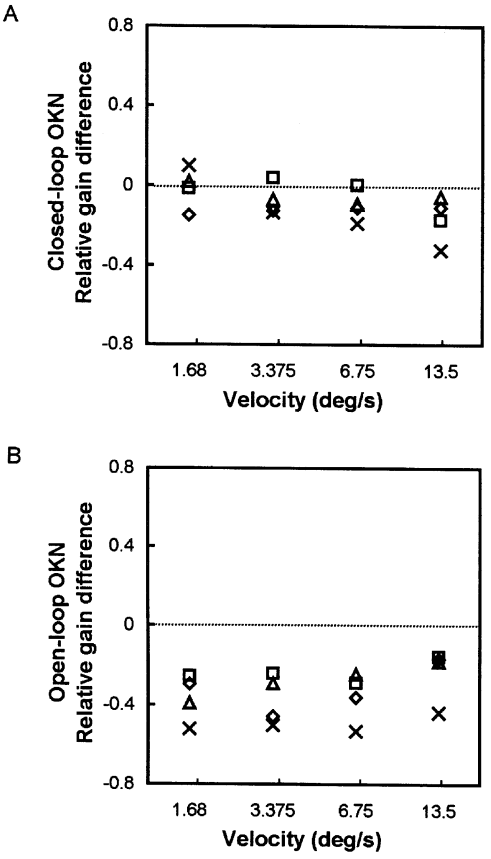

Fig. 2A, B Relative difference in gain of optokinetic nystagmus (OKN) after diazepam and placebo [$(G_d-G_p)/G_p$, G_d: OKN gain after diazepam, G_p: OKN gain after placebo]. The *dashed line* represents no difference. The four different symbols are for the four subjects. **A** Under the closed-loop condition; **B** under the open-loop condition

was quite stable after intake of either diazepam or placebo due to the strong negative feedback control of the OKN system. Relative gain differences of closed-loop OKN after diazepam and placebo for all subjects are plotted as the function of stimulus velocity in Fig. 2A. The data points were found to be distributed about the dashed line at all but the highest velocity tested. One of the four subjects showed a relatively large difference at 13.5 deg/s. Probably this subject was extraordinarily sensitive to the diazepam treatment. However, statistical analysis showed that no significant drug effect upon closed-loop gain after diazepam was found when compared to gain after placebo ($P = 0.92$; $P = 0.16$; $P = 0.09$; $P = 0.08$ for the four velocities, respectively). This suggests that the closed-loop OKN gain is not sensitive to the effect of diazepam.

Open-loop OKN responses

Samples of OKN eye movement under the open-loop condition are illustrated in Fig. 1B. As is shown in this figure, the slope of the slow phase of OKN after diazepam was flatter than that after placebo, which implies that the open-loop OKN gain decreased after diazepam.

The OKN gain under open-loop condition after diazepam and placebo for the four subjects is presented in Table 2. Under open-loop condition, the OKN gain was much larger than 1 at low stimulus velocities. Relative gain differences of open-loop OKN after diazepam and placebo for all subjects are plotted as the function of

Table 2 Open-loop OKN gain after administration of diazepam and placebo

Subject	1.68 deg/s		3.375 deg/s		6.75 deg/s		13.5 deg/s	
	Placebo	Diazepam	Placebo	Diazepam	Placebo	Diazepam	Placebo	Diazepam
1	4.06	2.86	4.71	2.57	3.83	2.45	2.38	1.98
2	3.61	2.68	2.57	1.95	1.78	1.27	1.20	1.01
3	3.63	2.21	3.17	2.24	2.20	1.66	1.34	1.10
4	6.15	2.95	3.27	1.64	1.76	0.83	1.01	0.57

velocity in Fig. 2B. All data points were found to be located below the dashed line. Paired *t*-tests indicate that diazepam significantly reduced open-loop OKN gain at all test velocities when compared to gain after placebo ($P = 0.02$; $P = 0.01$; $P = 0.01$; $P = 0.007$ for the four velocities, respectively). This suggests that the open-loop OKN gain is more sensitive to pharmacological effects.

Discussion

In a double-blind, placebo-controlled study, we investigated the effect of diazepam on closed- and open-loop OKN eye movements. Our results show that diazepam reduced the gain of open-loop OKN, while having no significant effect on the gain of closed-loop OKN. These results suggest that the open-loop OKN gain could be used as a sensitive measure of the effects of Bzs.

Advantage of open-loop OKN in evaluating drug effects

Optokinetic responses have traditionally been described as negative feedback control systems (Collewijn 1981; Frens et al. 2000; Leigh and Zee 1999). For a negative feedback system, if its open-loop gain (or the internal amplification factors) is G_{OL}, the closed-loop gain, G_{CL}, could be theoretically calculated by the equation $G_{CL} = G_{OL}/(1 + G_{OL})$. Obviously, when G_{OL} is largely greater than 1, the closed-loop gain G_{CL} is close to 1 regardless of the varying of G_{OL}. Therefore, negative feedback offers a stable response to stimuli and a relative insensitivity to changes in internal parameters (Leigh and Zee 1999). Indeed, in the present experiments, the closed-loop gain of OKN was always found to be roughly close to 1 even if pharmacological action occurred in its neural pathway. However, the open-loop gain was distinctly reduced when the CNS was suppressed by diazepam. This indicates that measuring the open-loop response directly is a more sensitive way of determining if there has been a change in the internal workings of the OKN system.

Specific effects of diazepam on OKN eye movements

It is known that γ-aminobutylic acid (GABA) is one of the two major inhibitory neurotransmitters in the brain, and Bzs seem to potentiate GABAergic inhibition in the central nervous system. Neurophysiologic results have revealed that Bzs bind densely in the frontal and occipital cortical areas and in vermal and floccular cerebellar areas (Möhler and Okada 1978; Speth et al. 1978), occupying binding sites in brain areas important to oculomotor control. Therefore, a knowledge of how these drugs modulate the OKN in humans could shed light on the organization of the system and provide tools for manipulating it experimentally or clinically. OKN eye movements are the result of a complex visuo-oculomotor transformation process, which involves many structures at the cortical as well as cerebellar and brainstem level (Collewijn 1981; Ilg 1997). It is generally assumed that the nerve cells in the inferior olive form part of the climbing fiber within the cerebellum and provide visual movement inputs (retinal slip signals) (Behrens et al. 1989; Frens et al. 2000). Thus, dysfunction in the cerebellum could affect OKN gain, especially under the open-loop condition. The diazepam-induced eye movement changes reported here may be explained by the disruption of sensory-motor contributions within the cerebellum that mediate eye movements.

The alteration in OKN slow phase velocity observed in the present study was similar to that observed in smooth pursuit velocity (Rothenberg and Selkoe 1981b). These eye-movement results provide direct objective evidence that after administration of a single therapeutic dose of commonly used benzodiazepine compounds, significant impairment in oculomotor control is present. Both smooth pursuit and optokinetic systems contribute to the stabilization of images of moving objects. In such cases, the tracking of objects moving across the visual fields, as well as the quality of vision, may be impaired to a certain degree. The wide usage of tranquilizers today for a variety of medical and psychiatric purposes underscores the importance of the clear documentation of such drug effects. Our results suggest that OKN eye movements, especially under the open-loop condition, provide a sensitive and objective method for assessing the sedative effect of drugs in humans.

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References

Behrens F, Grüsser O-J, Roggenkämper P (1989) Open-loop and closed-loop optokinetic nystagmus in squirrel monkeys (*Saimiri sciureus*) and in man. Prog Brain Res 90:183–196

Bittencourt PRM, Wade P, Smith AT, Richens A (1983) Benzodiazepines impair smooth pursuit eye movements. Br J Clin Pharmacol 15:259–262

Bollen E, Bax J, van Dijk JG, Koning M, Bos JE, Kramer CGS, van der Velde EA (1993) Variability of the main sequence. Invest Ophthalmol Vis Sci 34:3700–3704

Collewijn H (1981) The optokinetic system. In: Zuber BL (eds) Models of oculomotor behavior and control. CRC Press, Florida, pp 112–139

Frens MA, Mathoera AL, van der Steen J (2000) On the nature of gain changes of the optokinetic reflex. Prog Brain Res 124:247–255

Hopfenbeck JR, Cowley DS, Radant A, Greenblatt DJ, Roy-Byrne PP (1995) Effects of diphenhydramine on human eye movements. Psychopharmacology 118:280–286

Ilg UJ (1997) Slow eye movements. Prog Neurobiol 53:293–329

Leigh RJ, Zee DS (1999) The neurology of eye movements, 3rd edn. Oxford University Press, New York

Matsunaga T, Shiraishi T, Kubo T (1983) Differential effects of diazepam upon vestibulo- and visual-oculomotor responses in the rabbit. Acta Otolaryngol Suppl 393:33–39

Möhler H, Okada T (1978) Biochemical identification of the site of action of benzodiazepines in human brain by ^{3}H-diazepam binding. Life Sci 22:985–996

Robinson DA (1963) A method of measuring eye movement using a scleral search coil in a magnetic field. IEEE Trans Biomed Electr 10:137–145

Rothenberg S, Selkoe D (1981a) Specific oculomotor deficit after diazepam. I. Saccadic eye movements. Psychopharmacology 74:232–236

Rothenberg S, Selkoe D (1981b) Specific oculomotor deficit after diazepam. II. Smooth pursuit eye movements. Psychopharmacology 74:237–240

Roy-Byrne PP, Cowley DS, Radant A, Hommer D, Greenblatt DJ (1993) Benzodiazepine pharmacodynamics: utility of eye movement measures. Psychopharmacology 110:85–91

Salas C, Navarro F, Torres B, Delgado-Garcia JM (1992) Effects of diazepam and D-amphetamine on rhythmic pattern of eye movements in goldfish. Neuroreport 3:131–134

Speth RC, Wastek GJ, Johnson PC, Yamamura HI (1978) Benzodiazepine binding in human brain: characterization using (^{3}H) flunitrazepam. Life Sci 22:859–866

Tian J, Yang Q, Wei M, Terdiman J, Sun F (2002) Neostigmine-induced alterations in fast phase of optokinetic responses in myasthenic ocular palsies. J Neurol 249:867–874

Yang Q, Wei M, Sun F, Tian J, Chen X, Lu C (2000) Open-loop and closed-loop optokinetic nystagmus (OKN) in myasthenia gravis and nonmyasthenia subjects. Exp Neurol 166:166–172