Saccadic Characteristics of Monozygotic and Dizygotic Twins Before and After Alcohol Administration

B. W. Gale, L. A. Abel,* J. C. Christian,† J. Sorbel,† and R. D. Yee**

Purpose. To evaluate the degree of heritability in the latency, accuracy, and peak velocity of reflexive saccades in young adult monozygotic (MZ) and dizygotic (DZ) twins before and after the administration of a single dose of ethanol.

Methods. Saccades were recorded using a scleral search coil before and after alcohol consumption, and data were analyzed offline. Estimates of heritability based on intraclass correlations (ICCs) and using a maximum likelihood estimates of genetic variance were calculated for the saccadic measures made before and after alcohol, as well as for the changes in latency, accuracy, and velocity.

Results. Intraclass correlations for MZ twins (rMZ) were highly significant; those for DZ twins (rDZ) were not significantly different from zero. This disparity between rMZ and rDZ suggests either multiple gene interactions or in utero environmental differences in the MZ twins. Alcohol significantly prolonged latency, reduced accuracy, and lowered peak velocity. Although the changes after alcohol were not significant, heritability values increased in all three measures after alcohol administration.

Conclusions. Latency, accuracy, and peak velocity appear to be controlled by multiple genes or to depend on prenatal environmental factors. Even a single low dose of alcohol appeared to enhance heritability measures. Differences seen between ICCs for latency, accuracy, and velocity after alcohol administration suggest that developmental control of the neural mechanisms underlying each measure may vary. Invest Ophthalmol Vis Sci. 1996;37:339–344.

The quantitative examination of ocular motor function has proven to be a useful tool for evaluating the mechanisms of a wide range of neurologic, ophthalmologic, and psychiatric disorders, as well as assessing the effects of aging and drugs.[1–6] An observation in such studies has been that the range of performance that must be considered normal is broad. For example, maximum saccadic velocity may vary from 400°/second to more than 800°/second.[1] Attempts to determine whether ocular motor performance is genetically or environmentally determined have been confined largely to the assessment of smooth pursuit in psychiatric populations,[7–10] although some saccadic characteristics have been studied.[11] These investigations have been carried out in either monozygotic (MZ) and dizygotic (DZ) twin pairs or in patients and their first-degree relatives. Recently, we reported[12] the results of a pilot study in a group of eight MZ twin pairs, in whom intraclass correlations (rMZ) were computed for saccadic latency, accuracy, and asymptotic peak velocity, as well as smooth pursuit gain. Significant rMZs were found for all measures. Although study of MZ twins alone can say nothing about the relative contribution of genetic versus environmental influences, the high correlations found suggested that a larger study, including both types of twins, was merited.

Although only limited research has been conducted on the sources of variability in normal ocular motor behavior, even less has been conducted on the determinants underlying the changes in eye movements caused by drugs. In particular, alcohol has been found to lead to marked decrements on performance of both the saccadic[4,5,13,14] and smooth pursuit[4,15–17] systems. However, there have been no twin studies

*From the Departments of *Ophthalmology and †Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana.*
Supported by United States Public Health Service grant P50 AA07611 to T-K Li and by an unrestricted grant from Research to Prevent Blindness, Inc. to the Department of Ophthalmology, Indiana University School of Medicine.
Submitted for publication May 22, 1995; revised October 23, 1995; accepted October 24, 1995.
Proprietary interest category: N.
Reprint requests: Larry Abel, Department of Ophthalmology, Indiana University School of Medicine, 702 Rotary Circle, Indianapolis, IN 46202-5175.

addressing the relative environmental and genetic influences on ocular motor deficits caused by alcohol. Other physiological characteristics have been examined in this fashion, however. Two twin studies on the electroencephalogram[18] and psychomotor performance[19] before and after alcohol ingestion suggested that these parameters, as well as the effect of alcohol on them, are all under strong influence of genetic interactions. In the current article, we examined saccadic latency, peak velocity, and accuracy in MZ and DZ twins to evaluate the genetic influences on the control of saccadic eye movements both before and after consumption of alcohol.

METHODS

Twenty-four pairs of MZ twins were tested (mean ± SD, 33.6 years ± 8.3 years). There were 10 male and 14 female pairs. Also tested were 21 pairs of same-sex DZ twins (32.1 years ± 8.2 years), with 11 male and 10 female pairs. All subjects were social drinkers who had no known neurologic or eye-movement disorders. The research was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board, with written informed consent obtained from subjects after the procedures were explained to them. Two hours after a standard light breakfast, the subjects were administered 0.44 ml/kg ethanol for male twins and 0.41 ml/kg for female twins, in an 11.88% solution with caffeine-free diet soda. Drinks were divided into five equal portions, and the subjects were given two minutes to drink for each portion. Dosages were selected to bring the subjects to a blood alcohol concentration of 0.05%. Breath alcohol was measured immediately before and after the post-alcohol eye movement studies using a breathalyser (Datamaster Breath Alcohol Analyzer; National Patent Analytical System, Mansfield, OH).

Horizontal saccadic eye movements of the dominant eye were recorded while subjects viewed the target binocularly. Testing was carried out both before and immediately after ethanol ingestion. Because of the time constraints imposed by the use of the search coil and the performance of the same test sequence before and after alcohol ingestion, vertical eye movements were not examined, although an uncalibrated vertical position signal was available on the chart recording for identifying blinks when the horizontal position data were ambiguous. Subjects were seated in a chair with a head rest and forehead brace. The magnetic search coil method was used for recording.[20] The target was produced by an 0.5-mW helium–neon laser and rear-projected by a computer-controlled mirror galvanometer onto the opposite side of a translucent screen 1 m in front of the subjects. Subjects were instructed to follow the target only with their eyes, keeping the head still. The test sequence was composed of three consecutive trials of target jumps with amplitudes of 5°, 10°, and 15° to and from the center position, with both amplitude and interstimulus intervals randomized. There were 23 trials at 5° and 18 trials each at 10° and 15°. After antialiasing filtering at 200 Hz, horizontal eye movement signals were digitized at 400 Hz for subsequent analysis, which was performed off-line using an interactive program. It used a high threshold (nominally 50°/second) to approximate the beginning and end of the saccade, after which the operator zoomed in on the saccade and adjusted the cursors by eye to make the final selection. This process also allowed the operator to discard saccades that occurred during blinks.

Data were analyzed using the TWINAN90 program.[21] To obtain unbiased estimates of heritability using the twin model, a number of assumptions are required. These assumptions and their potential effects on the results are:

1. The data are normally distributed within zygosities. As part of TWINAN90,[21] the data were tested, by zygosity, for departures from normality, and a transformation that normalized the zygosity distributions was recommended.
2. The twins are a random sample of population twins. The means of the MZ and DZ twins were compared to search for evidence of biased samplings.
3. There is random mating within the population. Positive assortative mating in the population increases the genetic similarity of DZ twins and, if present, causes an underestimate of genetic effects.
4. Total variances of MZ and DZ twins are equal. Greater MZ total variance tends to bias downward estimates of genetic effects, whereas greater DZ total variance tends to bias upward estimates of genetic effects. The variances of MZ and DZ twins were compared using an F′ test.[22]
5. Environmental covariance of MZ twins equals the environmental covariance of DZ twins. If the environmental covariance of MZ twins is greater than the environmental covariance of DZ twins, estimates of genetic effects will be biased upward.

MZ and DZ intraclass correlations (rMZ and rDZ) were calculated and tested for significance as described by Snedecor and Cochran.[23] The following formula was used to calculate rMZ and rDZ, using the among-pair mean squares (AMS) and within-pair mean squares (WMS):

$$\text{intraclass correlation} = \frac{(\text{AMS} - \text{WMS})}{(\text{AMS} + \text{WMS})}$$

TABLE 1. Latency

Target Amplitude (°)	*Mean MZ (sec^{-1})*	*rMZ*	*Mean DZ (sec^{-1})*	*rDZ*	*ICC H^2*	*MLE H^2*
Before Alcohol (N_{MZ} = 24, N_{DZ} = 21)						
5	5.23	0.541*	5.31	0.179	0.725 (0.17)	0.54 (0.14)
10	5.07	0.379†	5.34	0.112	0.535	0.39
15	4.45	0.676*	4.7	0.187	0.978†	0.677†
After Alcohol (N_{MZ} = 24, N_{DZ} = 21)						
5	4.81‡	0.692*	4.94‡	0.272	0.84 (0.066)	0.748*
10	4.77‡	0.745*	4.92‡	0.138	1.215*	0.756*
15	4.18‡	0.694*	4.38‡	0.35	0.689 (0.12)	0.717†

* $P < 0.01$; † $P < 0.05$; ‡ $P < 0.001$.
ICC = interclass correlation; MLE = maximum likelihood estimator; H^2 = heritability.

Twins are a special case of a one-way, random effects, analysis of variance with two subjects in each group. The expected values of the intraclass correlations are a ratio of genetic and environmental covariances divided by the total variance. However, as MZ cotwins are genetically identical and DZ cotwins share, on the average, half of their genes in common, the difference (rMZ–rDZ) is expected to be the result of genetic covariance if the assumptions of the twin model hold.

The null hypotheses that rMZ and rDZ = 0 were tested, after the method described by Snedecor and Cochran[23] using one-tailed F-ratios (AMS/WMS). If the additional assumption is made that all genetic variance is additive, heritability, or that fraction of total variance caused by genetic variance, may be estimated by the formula 2(rMZ − rDZ). The null hypothesis that rMZ = rDZ was tested by comparing Z transformations of rMZ and rDZ.

RESULTS

For latency and accuracy, the results were separated by target amplitude into three groups. Because saccades are often somewhat inaccurate and peak velocity is more strongly dependent on saccade amplitude than are latency and accuracy, peak velocity results were grouped by saccade rather than by target amplitude. Responses were separated into three bins, each 2° wide. This was a compromise between including relatively homogeneous data in each bin and not excluding from analysis an excessive number of responses. These bins included saccades between 4° and 6°, 7° and 9°, and 10° and 12°.

Before statistical analyses, data were examined using the Kolmogorov–Smirnov test to determine whether groups were distributed normally. Because of violations of the assumption of a normal distribution, the latency and accuracy results were normalized by inverse and square transformations, respectively. Although there were also violations from normality in the pre-alcohol peak velocity data for MZ twins at low and middle amplitude bins, no suitable data transformation could be found. Hence, the analysis of peak velocity was performed with the original data. The effect of alcohol was obtained by subtracting the pre-alcohol values from post-alcohol values (using the original data). Distributions of the resultant variables

TABLE 2. Accuracy

Target Amplitude (°)	*Mean MZ (accuracy²)*	*rMZ*	*Mean DZ (accuracy²)*	*rDZ*	*ICC H^2*	*MLE H^2*
Before Alcohol (N_{MZ} = 24, N_{DZ} = 21)						
5	0.69	0.737*	0.72	0.32	0.834†	0.677 (0.15)
10	0.69	0.714*	0.69	0.321	0.787 (0.07)	0.679 (0.08)
15	0.68	0.684*	0.67	0.382†	0.604 (0.16)	0.656
After Alcohol (N_{MZ} = 24, N_{DZ} = 21)						
5	0.64‡	0.615*	0.65‡	0.023	1.184†	0.527 (0.08)
10	0.64‡	0.565*	0.68	−0.115	1.361†	0.455†
15	0.66	0.516*	0.67	0.192	0.649	0.505

* $P < 0.01$; ‡ $P < 0.05$; ‡ $P < 0.001$.
ICC = interclass correlation; MLE = maximuim likelihood estimator; H^2 = heritability.

TABLE 3. Peak Velocity

Saccade Amplitude (°)	*Mean MZ (°/s)*	*rMZ*	*Mean DZ (°/s)*	*rDZ*	*ICC H^2*	*MLE H^2*
Before Alcohol (N_{MZ} = 22, N_{DZ} = 20)						
4–6	212.4	0.502*	218.3	0.002	1.00†	0.413 (0.16)
7–9	296.7	0.704*	306.3	0.263	0.882†	0.579
10–12	335.8	0.717*	344.7	−0.019	0.143†	0.60 (0.11)
After Alcohol (N_{MZ} = 22, N_{DZ} = 20)						
4–6	204.2‡	0.700*	205.8§	0.066	1.27*	0.67*
7–9	271.1§	0.790*	283.6§	0.123	1.33*	0.69 (0.074)
10–12	308.1§	0.894*	319.1§	−0.128	1.79*	0.80†

* $P < 0.01$; † $P < 0.05$; ‡ $P < 0.0001$; § $P < 0.02$.
ICC = interclass correlation; MLE = maximuim likelihood estimator; H^2 = heritability.

were not significantly different from normal. Peak velocity also was found to have significantly greater MZ than DZ variance for 7° to 9° and 10° to 12° amplitudes, both before and after alcohol administration. This finding may be related to the inability to find an appropriate normalizing transformation. Blood alcohol concentrations, estimated from breath analysis, were 0.061% (SE = 0.003%) and 0.051% (SE = 0.003%) before and after measurement of the eye movements, respectively.

Means, summed mean squares, and intraclass correlation coefficients (ICC) rMZ and rDZ were computed for all variables. Heritability was first estimated with the ICCs using the formula $H^2 = 2(rMZ - rDZ)$.[24] This definition of heritability requires that genetic variance be additive, with no gene interactions. Also used was a maximum likelihood estimation model of heritability, wherein variance was apportioned into error (E), additive genetic (A), and common environmental (C) components. The difference in fit between the ACE and CE models was compared.[25] Results are shown in Tables 1, 2, and 3 for latency, accuracy, and peak velocity, respectively (velocity measures were only carried out for 22 MZ and 20 DZ twin pairs because of noise problems with data for three twin pairs). Note that all 18 rMZs, but only 1 rDZ, reached significant levels. Because significance levels of the heritability estimates appeared to be limited by sample size, Tables 1 to 3 include not only those heritability estimates found to be significant but also those in which a trend appeared to be present. The intraclass correlation estimate of H^2 heritability estimates exceeded 1 in one of the latency, two of the accuracy, and five of the velocity measures, suggesting the presence of a genetic interaction term. Maximum likelihood estimation estimates of heritability were, by nature, less than 1. A number of covariates were associated with saccadic accuracy and peak velocity, though not with latency (Table 4).

The effects of alcohol on saccadic performance were highly significant for all but 3 of 18 possible combinations of variable, amplitude, and zygosity, as noted in Tables 1 to 3. Alcohol increased latency, decreased accuracy, and reduced peak velocity. This was consistent with previous studies on the effects of alcohol on the saccadic system.[4,5,13,14] However, the heritability estimates for these changes were all nonsignificant. Heritability estimates for the pre- and post-alcohol ingestion data themselves were often of borderline significance for our sample sizes, a finding likely caused by their derivation from second-order statistics. To find statistically significant heritability in measures of changes in these variables would require even larger samples.

DISCUSSION

Our results extend our pilot findings on saccadic concordance in MZ twins.[12] The addition of DZ twins has enabled us to make several estimates of the degree of genetic influence on saccadic function, both before and after ingestion of a low dose of alcohol. The pattern of results was somewhat complex and differed among the three parameters examined. Although the differences seen between performance before and after alcohol ingestion showed no evidence of heritability, there was a pattern of more variables appearing heritable after alcohol ingestion. This resembles the findings observed for auditory event-related potential components.[26] Alcohol did not have the same effect on the intraclass correlations for all three measures, however. For latency, rMZ and rDZ both increased after alcohol ingestion; for peak velocity, rMZ increased, whereas rDZ remained near zero. For accuracy, rMZ declined somewhat and rDZ approached zero. The disparity between relatively high rMZ and near zero rDZ could reflect the interaction of two or more genes in the determination of the measures under study. Alternatively, the possibility exists that these correlations reflect inequalities in the common envi-

TABLE 4. Covariates for Saccadic Accuracy and Peak Velocity

	Covariates (r^2) Before Alcohol	*Covariates (r^2) After Alcohol*
Accuracy		
5° target	Age (−0.25)	Age (−0.18), height (−0.08)
10°	Age (−0.28), drinking (−0.13)	Age (−0.19), drinking (−0.06)
15°	Age (−0.11), drinking (−0.09)	Age (−0.12)
Peak velocity		
4–6° saccades	Weight (0.06)	Drinking (−0.12)
7–9	Age (−0.06)	
10–12°	Age (−0.09)	

ronmental variance within the MZ pairs—e.g., in placental and amniotic structure.[25] These differences could produce high rMZ values in traits that are independent of genetic influence. Confirmation of this would require the comparison of MZ twins of known placental types.

Saccadic latency in this context is essentially a simple reaction time task; its value depends on the level of performance of all the stages involved, from processing the visual input to generating the motor command. For latency, the ratio H^2/rMZ was greater than 1 for all three amplitudes, indicating a failure of one of the underlying assumptions of the statistical model.[21,24] This remained true after alcohol administration, with both intraclass correlations and heritability measures increasing, suggesting that after alcohol ingestion, the pathways that trigger reflexive saccades appear to be even more strongly under genetic influence. For accuracy, the picture is different. Before alcohol ingestion, H^2/rMZ was less than 1 for all target amplitudes, suggesting that the assumptions of the intraclass correlation heritability model were at least not grossly violated. After alcohol ingestion, all intraclass correlations dropped, but the change was most marked for rDZs. This led to H^2 values greater than 1, with H^2/rMZ reaching 2. Clearly, the simple additive variance model does not fit these data well. The modest declines after alcohol seen in rMZ and the drop of rDZ to near zero suggest either that strong genetic interactions are present and that changes in accuracy after alcohol ingestion depend on these all being identical or that differences in common environmental influences within MZ twin pairs influence this measure. For peak velocity, the simple additive heritability model appears to fail before and after alcohol ingestion.

The modest covariations of accuracy and velocity with age and drinking history were unexpected. Previous studies of age-related impairment of saccadic function have observed only modest age-related declines in the elderly,[1,27] making the reductions noted in accuracy and peak velocity in our relatively young subjects unexpected. This may be a function of the increased sensitivity provided by our relatively large sample size. A history of drinking as a modest cofactor, largely for accuracy, is also unexpected in a population screened to exclude individuals with a history of alcohol abuse. Although this could reflect subclinical changes in the cerebellum or other regions involved in computing retinal error, a larger study focused on this issue is necessary for resolution of the question.

Key Words

alcohol, genetics, saccade, twin

References

1. Abel LA, Troost BT, Dell'Osso LF. The effects of age on normal saccadic characteristics and their variability. *Vision Res.* 1983;23:33–37.
2. Iacono WG, Peloquin LJ, Lumry AE, Valentine RH, Tuason VB. Eye tracking in patients with unipolar and bipolar affective disorders in remission. *J Abnorm Psychol.* 1982;91:35–44.
3. Abel LA, Hertle RW. Effects of psychoactive drugs on ocular motor behavior. In: Johnston CW, Pirozzolo FJ, eds. *Neuropsychology of Eye Movements.* Hillsdale, NJ: Lawrence Erlbaum Associates; 1988:81–114.
4. Baloh RW, Sharma S, Moskowitz H, Griffith R. Effects of alcohol and marijuana on eye movements. *Aviat Space Environ Med.* 1979;50:18–23.
5. Jantti V, Lang AH, Keskinen E, Lehtinen I, Pakkanen A. Acute effects of intravenously given alcohol on saccadic eye movements and subjective evaluations of intoxication. *Psychopharmacology.* 1983;79:251–255.
6. Bittencourt PRM, Wade P, Smith AT, Richens A. The relationship between peak velocity of saccadic eye movements and serum benzodiazepine concentration. *Br J Clin Pharmacol.* 1981;12:523–533.
7. Holzman PS, Solomon CM, Levin S, Waternaux CS. Pursuit eye movement dysfunctions in schizophrenia: Family evidence for specificity. *Arch Gen Psychiat.* 1984;41:136–139.
8. Holzman PS. The use of eye movement dysfunction in exploring the genetic transmission of schizophrenia. *Eur Arch Psychiatr Neurol Sci.* 1989;239:43–48.
9. Clementz BA, Sweeney JA, Hirt M, Haas G. Pursuit gain and saccadic intrusions in first-degree relatives of probands with schizophrenia. *J Abnorm Psychol.* 1990;99:327–335.
10. Iacono WG, Clementz BA. A strategy for elucidating

genetic influences on complex psychpathological syndromes (with special reference to ocular motor functioning and schizophrenia). *Prog Exp Personality Psychopath Res.* 1993;16:11–65.
11. Iacono WG, Lykken DT. Eye tracking and psychopathology: New procedures applied to a sample of normal monozygotic twins. *Arch Gen Psychiatry.* 1979; 36:1361–1369.
12. Bell BB, Abel LA, Li W, Christian JC, Yee RD. Concordance of smooth pursuit and saccadic measures in normal monozygotic twin pairs. *Biol Psychiatry.* 1994;36:522–526.
13. Lehtinen I, Lang AH, Jantti V, Keskinen E. Acute effects of alcohol on saccadic eye movements. *Psychopharmacology.* 1979;63:17–23.
14. Tedeschi G, Quattrone A, Bonavita V. Saccadic eye movements analysis as a measure of drug effect on central nervous system function. *Ital J Neurol Sci.* 1986;7:223–231.
15. Kobatake K, Yoshii F, Shinohara Y, Nomura K, Takagi S. Impairment of smooth pursuit eye movement in chronic alcoholics. *Eur Neurol.* 1983;22:392–396.
16. Flom MC, Brown B, Adams AJ, Jones RT. Alcohol and marijuana effects on ocular tracking. *Am J Optom Physiol Optics.* 1976;53:764–773.
17. Lehtinen I, Nyrke T, Lang AH, Pakkanen A, Keskinen E. Quantitative effects of ethanol infusion on smooth pursuit eye movements in man. *Psychopharmacology.* 1982;77:74–80.
18. Christian JC, Li T–K, Norton JA, Propping P, Yu P–L. Alcohol effects on the percentage of beta waves in the electroencephalograms of twins. *Genet Epidemiol.* 1988;5:217–224.
19. Martin NG, Oakeshott JG, Gibson JB, Starmer GA, Perl J, Wilks AV. A twin study of psychomotor and physiological responses to an acute dose of alcohol. *Behav Genet.* 1985;15:305–347.
20. Collewijn H, Van der Mark F, Jansen TC. Precise recording of human eye movements. *Vision Res.* 1975;15:447–450.
21. Williams CJ, Christian JC, Norton JA. TWINAN90: A FORTRAN program for conducting ANOVA-based and likelihood-based analyses of twin data. *Comp Methods Program Biomed.* 1992;38:167–176.
22. Christian JC, Kang KW, Norton JA. Choice of an estimate of genetic variance from twin data. *Am J Hum Genet.* 1974;26:154–161.
23. Snedecor GW, Cochran WG. *Statistical Methods.* 6th ed. Ames, IA: Iowa State University Press; 1967.
24. Slemenda CW, Christian JC, Williams CJ, Norton JA, Johnston CC. Genetic determinants of bone mass in adult women: A reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. *J Bone Mineral Res.* 1991;6:561–567.
25. O'Connor SJ, Morzorati S, Christian JC, Li T–K. Heritable features of the auditory oddball event-related potential: Peaks, latencies, morphology and topography. *Electroencephal Clin Neurophysiol.* 1994;92:115–125.
26. O'Connor SJ, Morzorati SL, Christian JC, Li T–K, Sorbel JJ. Effects of alcohol on heritable features of the ERP. *Alcohol: Clin Exp Res.* 1994;18:450. Abstract.
27. Warabi T, Kase M, Kato T. Effect of aging on the accuracy of visually guided saccadic eye movement. *Ann Neurol.* 1984;16:449–454.