

Effects of Methanol on the Retinal Function of Juvenile Rats

C. Plaziac¹, P. Lachapelle², C. Casanova^{1,*}

¹Laboratoire des Neurosciences de la vision, École d'optométrie, Université de Montréal, Montreal, Que., Canada H3C 3J7

²Department of Ophthalmology, McGill University, Montreal, Que., Canada

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Abstract

We have investigated the effect of methanol exposure on the retinal function of juvenile rats. The electroretinogram (ERG) and oscillatory potentials (OPs) were recorded prior to and up to 72 h after the administration of methanol. Data were compared to a control group which was only exposed to physiological saline. Our findings can be summarized as follows: methanol generally reduced the amplitude of all retinal potentials, and in some cases, to baseline levels. The ERG b-wave was affected earlier and more prominently than the a-wave. All measured OPs (2–4) were decreased but OP2 was less affected, suggesting that the cone pathway may be less sensitive to methanol than the rod-mediated pathway. These data indicate that juvenile rats (21 days old, i.e. with an immature synaptic development) present a sensitivity to methanol comparable to that observed in adult animals.

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INTRODUCTION

Methanol is used as a solvent in many products such as antifreeze, windshield fluid, varnish and gasoline. Accidental or intentional exposure to this alcohol can yield mild to severe health problems and, in extreme cases, coma and death (Ingemansson, 1984; Garner and Lee, 1994; Sullivan-Mee and Solis, 1998). The neurotoxicity of methanol (through acute, subacute or chronic poisoning) is attributed to its metabolite, formic acid or formate, which inhibits the cytochrome oxidase system, necessary for ATP production (Eells et al., 1996; McKellar et al., 1997). Humans are particularly sensitive to methanol because they have a limited capacity to rapidly oxidize and therefore eliminate formic acid (Snyder and Andrews, 1996).

Methanol is generally considered as a neurotoxin of the human visual system as it may provoke visual defects, from blurred vision to blindness, depending on the severity of the exposure (Sullivan-Mee and Solis, 1998; Seme et al., 1999). These dysfunctions are likely due to its action on the retina and the optic nerve. For instance, methanol was shown to provoke degeneration of retinal ganglion cells (Snyder and Andrews, 1996), the swelling of photoreceptors inner segments (Seme et al., 2001), the edema of the optic disk and the inhibition of electrical conduction along the optic nerve (Hayreh et al., 1977). At the functional level, methanol was shown to reduce the amplitude of the electroretinogram (ERG) of normal adult rats at lower formate concentrations than those required to diminish the flash-evoked cortical potentials (Eells, 1991; Murray et al., 1991; Garner and Lee, 1994; Lee et al., 1994; Eells et al., 1996; McKellar et al., 1997; Seme et al., 1999, 2001). The ERG thus represents a very sensitive means to study methanol-induced retinotoxicity. In the present study, we have used this electrophysiological approach to study the effects of methanol on the visual

Abbreviations: ERG, electroretinogram; OP, oscillatory potential

* Corresponding author. Tel.: +1-514-343-2407; fax: +1-514-343-2382.

E-mail address: christian.casanova@umontreal.ca (C. Casanova).

function of juvenile rats, our assumption being that these animals would exhibit increased susceptibility given the immaturity of their nervous system. We therefore recorded the ERG a-wave, b-wave as well as the oscillatory potentials (OPs) before and for 3 days following methanol administration.

MATERIALS AND METHODS

Experiments were carried out on 30 juvenile Long-Evans male rats aged 21 days at the onset of the experiment. Twenty-two animals were exposed to methanol and the remaining eight received physiological saline (control group). All animals were fed ad libitum and were subjected to a 12 h light/dark cycle. Animals were treated in accordance with the guidelines of the Canadian Council on Animal Care.

Methanol Exposure

Methanol (HPLC grade; Sigma) was diluted in sterile saline and was administered as a 20% weight/volume (w/v) solution. At the onset of the experiment, rats were placed in a hermetic Plexiglas chamber and exposed to N₂O/O₂ (1:1; flow rate 2 l/min) to render the animals sensitive to methanol (Eells et al., 1996). The first injection of methanol (i.p., 4 g/kg) was performed 4 h after being placed in the N₂O/O₂ environment (*T*₀). Supplemental doses of methanol were given at 12 h intervals (dose of 2 g/kg) for a total period of 72 h (*T*₂₄, *T*₄₈, and *T*₇₂). This regimen was previously shown in adult rats to yield blood formate concentration between 8 and 15 mM (Eells et al., 1996). Throughout the experiment, animals were only removed from the N₂O/O₂ environment to complete the electrophysiological recordings. The same protocol was used for the control group, but methanol was replaced by saline.

Electrophysiological Recordings

Animals were dark adapted for 12 h prior to the ERG recording. Animals were then removed from the N₂O/O₂ chamber and anesthetized by i.m. injection of a mixture of Ketamine (80 mg/kg), Atravet (0.04 mg/kg), and Atropine (0.5 mg/kg). Pupil dilatation was obtained with drops of 1% Atropine sulfate and 2.5% Phenylephrine. Proparacaine hydrochloride (Diocaine 0.5%) was used as a topical anesthetic. Carboxymethylcellulose sodium (Celluvisc, 1%) was used to protect the cornea from desiccation. The animals were placed in a light proof custom-made recording box.

Reproducible ERGs (bandwidth of 1–1000 Hz) and oscillatory potentials (100–1000 Hz) were recorded with a DTL fiber electrode placed on the cornea and each rat had its own DTL fiber (Hébert et al., 1999; Dembinska et al., 2001). Two subdermal electrodes were positioned on each animal's lower back and posterior part of the head to serve as ground and reference, respectively. ERG responses were generally recorded once but, in a few cases, represent an average of two to four flashes delivered 27 cm away from the cornea (Grass photostimulator, intensity of 16, $f = 0.1$ Hz).

After the recordings at *T*₇₂ were made, the rats were killed with an i.p. injection of about 0.5 ml of pentobarbital sodium (Euthanyl, 240 mg/ml). Data analysis included peak time and amplitude measurements taken prior to and at predetermined time intervals following methanol injection. Statistical significance was determined using one-way analysis of variance (ANOVA) with Dunnett's method ($P < 0.05$). Analyses were carried out with the software SigmaStat 2.0 (SPSS Science). For each recording, the relative amplitude of the OPs was determined by dividing the amplitude of a given OP by the sum of the three analyzed OPs.

RESULTS

The data reported below were obtained from 15 juvenile rats. Five of the 22 methanol-exposed animals had to be eliminated due to poor health status during the 3-day period. No reliable responses could be obtained from the remaining two rats due to extraneous noise. Overall, our data indicate that methanol has a profound effect on retinal potentials.

Representative examples are presented in Fig. 1. Panel A shows that the ERGs recorded 1 day before (control) and immediately after (*T*₀) the administration of methanol were comparable. In contrast, 24 h after the injection of methanol, the amplitude of the a-wave and b-wave were already decreased by 60 and 47% of control amplitude, respectively. Only the a-wave persisted at *T*₄₈, and no signal could be measured at *T*₇₂. A similar effect was observed for the amplitude of the major OPs (panel B) whereas their peak times were not significantly altered (panel B; $P < 0.05$, one-way analysis of variance). Analysis of individual records revealed that the ERG was virtually abolished in six animals, but showed slight persistence in the remaining nine (see panels C and D).

Individual data were averaged (as percent of control recordings made 1 day before the injection) and are

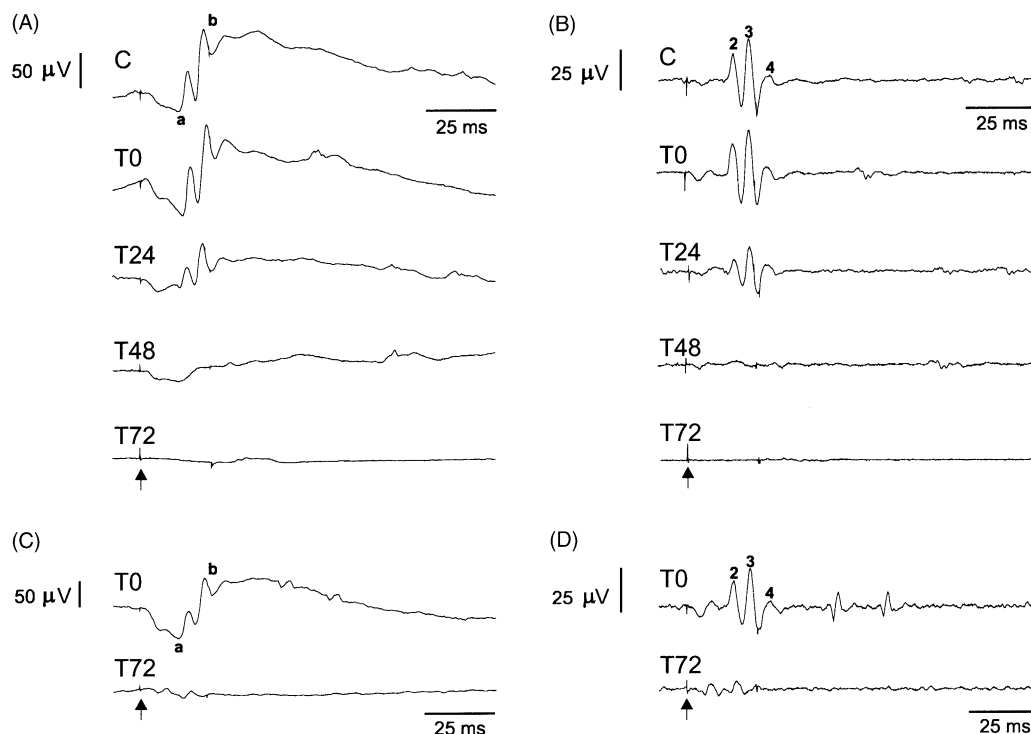


Fig. 1. Representative examples of the effects of methanol on retinal potentials. Panel A presents successive ERG recordings taken before (control), immediately after the initial administration of methanol (T_0), and throughout the following 72 h period (T_{24} – T_{72}). Panel B shows the corresponding OPs. In both cases, there was a total disappearance of the signals. Panels C and D illustrate cases in which there was a persistence of the ERG and OPs after 72 h. Some traces were contaminated by the ECG signal. Arrows represent flash onset. Abbreviation: C, control.

presented in Fig. 2. Panels A and B clearly illustrate that the amplitude of the a-wave and the b-wave were significantly decreased following the administration of methanol. When compared to the a-wave, the b-wave appeared to be more sensitive to methanol as it tended to be affected earlier (T_{24}) and its amplitude was more severely decreased (T_{72}). Panel C shows the amplitude of the ERG b-wave of the eight saline-exposed animals. The administration of physiological saline did not alter the retinal physiology and all potentials were comparable over time. This profile of action was significantly different than that observed with methanol (panel B; $P < 0.05$, one-way analysis of variance). The amplitude of the ERG b-wave of the control group tended to be lower at T_{48} and T_{72} , but was not significantly different from that computed at T_0 and T_{24} . Because the N_2O/O_2 environment per se does not appear to have a detrimental action on the retinal function (Seme et al., 1999, 2001), this lowering of amplitude may be related to a possible deterioration of the DTL electrode over time. This is unlikely because no significant change in the DTL fibers impedance is seen throughout short recording sessions (for discussions of the advantages and limits of the DTL electro-

des, see Hébert et al., 1995, 1999; Lachapelle et al., 1993). Another possibility is that this lowering of the signal came from a small-undetected cloudiness of the juvenile cornea associated with daily anesthetic procedures. Alternatively, these changes may be related to the on-going maturation of the nervous system and adjacent structures of the tested animals.

Fig. 3 presents the overall data for the OPs. One may note that the profile of the changes induced by methanol is very similar to that observed for the b-wave. As such, the effect became significant at T_{24} and responses barely survive at T_{72} (mainly for OPs 3 and 4). All measured OPs were affected by methanol ($P > 0.05$, one-way analysis of variance). However, comparing the relative amplitude of each OP revealed that OP2 was more resistant to methanol exposure (panels D–F).

DISCUSSION

Our study shows that the retinal physiology of juvenile rats is altered by the exposure to methanol. We present evidence that the amplitude of the a-wave,

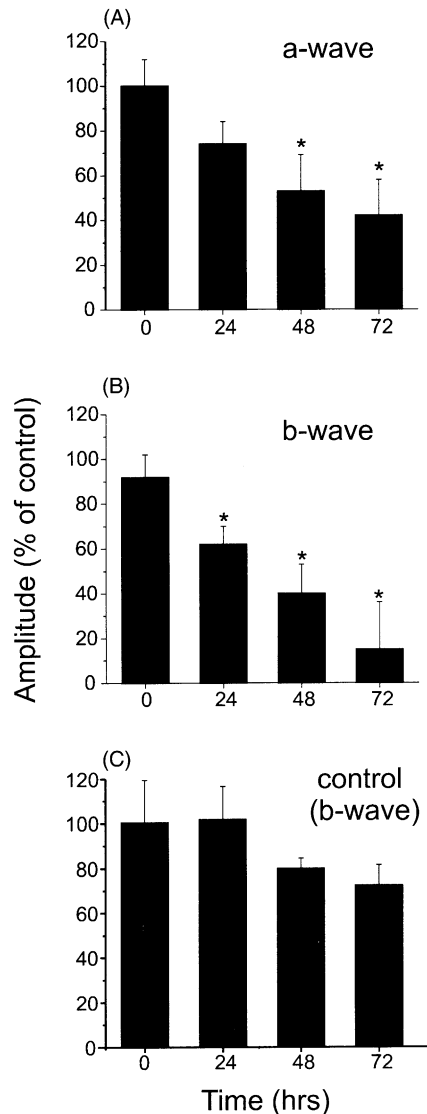


Fig. 2. Panels A and B present mean data obtained from the 15 methanol-exposed juvenile rats. Methanol provoked a significant decrease of the ERG a-wave and b-wave from T48 and T24, respectively. The asterisk indicates a significant decrease ($P < 0.05$). Panel C shows mean measurement of the b-wave amplitude for eight saline-exposed juvenile animals. No significant changes were observed during the whole testing period. Error bars represent S.E.M.

b-wave and OPs is severely decreased at 72 h following the administration of methanol; this diminution appearing earlier and being more pronounced for the b-wave and the oscillatory potentials than for the a-wave. This last observation is in agreement with the findings of Murray et al. (1991) showing that Müller cells are a direct target of methanol (through formate) and are more susceptible to its toxic effects than other cells. Since Müller cells are in part responsible for the generation of the b-wave (depolarization through a K^+ current loop; Stockton and Slaughter, 1989), the

latter should be the first potential to be affected by methanol exposure. Also, because OPs are closely linked to the b-wave (Gorfinkel and Lachapelle, 1990), it is not surprising that they behave in a comparable way when the retina is exposed to methanol.

Since a similar methanol dosing regime has been shown to provoke metabolic acidosis in adult rats (Eells et al., 1996), one may suggest that the retinal effects observed in juvenile animals may not be solely a direct consequence of formate but also a generalized effect from acidosis (Niemeyer and Steinberg, 1984; Larsson et al., 1985; Dawson et al., 1988). However, several observations support that the retinal defects reported in the present study were a direct consequence of formate action. Eells et al. (1996) reported that the use of a reduced methanol regimen (4–6 mM) that does not yield metabolic acidosis, still provoked a significant decrease of the ERG amplitude. Moreover, it has been recently demonstrated that formate specifically disrupt retinal mitochondrial energy production, reduces ATP and ADP production, and lowered the concentration of the retinal antioxidant glutathione (Seme et al., 2001). Acidosis was shown to spare the high frequency retinal potentials (Dawson et al., 1988), a result that is inconsistent with the loss of the oscillatory potentials reported here, and an increase of the c-wave amplitude (Niemeyer and Steinberg, 1984) which was not observed in the present study. Nevertheless, additional experiments including blood formate levels and pH measurements are necessary to clearly determine the contribution of acidosis to the visual defects provoked by methanol exposure in juvenile rats.

To our knowledge, this study is the first to investigate the effect of methanol in juvenile rats. Since these rats are still immature (stage 2 of the synaptogenesis; Lund and Lund, 1972), we postulated that they would be more sensitive than adult animals. This does not seem to be the case. A number of studies have examined the effect of methanol on the ERG a-wave and b-wave in the adult (Eells, 1991; Murray et al., 1991; Lee et al., 1994; Eells et al., 1996; McKellar et al., 1997; Seme et al., 1999, 2001). They found, as we did in juvenile rats, that the b-wave was more sensitive to methanol and was affected earlier than the a-wave. A similar conclusion can be reached with the OPs recordings. Garner and Lee (1994) studied the action of methanol on the OPs in adult rats and reported that all OPs were reduced (non-specific effect), even though their data suggest that OP1 and OP2 were the most affected. We found that OP3 and OP4 in juvenile rats were more sensitive to methanol than OP2. It is possible that the

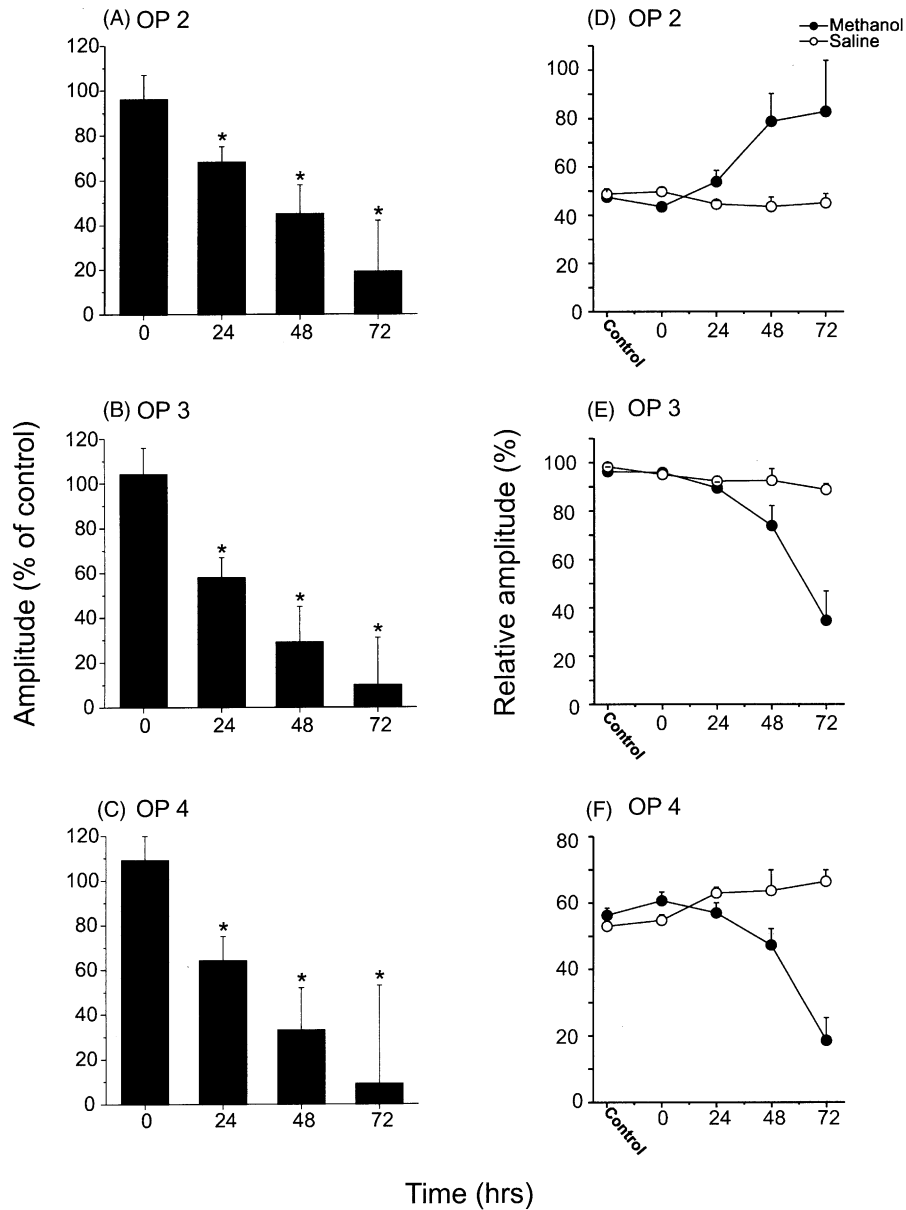


Fig. 3. Mean data obtained from the OPs of the 15 methanol-exposed juvenile rats. In all cases, methanol provoked a significant decrease of the amplitude of OPs 2–4 from T24. The asterisk indicates a significant decrease ($P < 0.05$). Error bars represent S.E.M. Panels D–F show the relative amplitude of each OP. Clearly, OP2 was less affected than OPs 3 and 4.

difference between both studies comes from the fact that Garner and Lee (1994) used a different method of analysis (OPs were measured by wavelet index method) than ours (Lachapelle et al., 1990). Of interest, it has been proposed that the short latency oscillatory potentials are generated through the cone pathway while activation of the rod pathway triggers the genesis of the longer latency OPs (King-Smith et al., 1986; Rousseau and Lachapelle, 1999). The fact that the short latency OP2 was less affected in our study would indicate that the cone pathway was more resistant to methanol exposure.

In conclusion, methanol-induced toxicity has comparable effects on the retinal physiology of juvenile and adult rats. It would be of interest to determine the level of recovery of the retinal function of methanol-exposed juvenile animals. A recent study (Seme et al., 2001) showed that adult rats can partially recover rod-dominated ERG responses 3 days after a methanol intoxication, but could not recover the cone-mediated ERG. The impact of the modes of treatment and administration should also be investigated based on the fact that trichloroethylene, a multipurpose neurotoxic solvent, respectively decreased and increase the

rabbit b-wave amplitude after being injected (acute experiments, Blain et al., 1990) and inhaled (chronic experiments, Blain et al., 1994). Finally, it remains to determine whether younger juvenile rats (from 14–15 days, when the eyes open, to 20 days) present a sensitivity to methanol comparable to the 21–24-day-old animal group investigated in the current study.

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