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THIP, a Selective Gamma-Aminobutyric Acid Receptor Agonist, Alters Flash-Evoked Potentials in Rats

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HETZLER, B. E. AND H. L. ZEISSET. *THIP, a selective gamma-aminobutyric acid receptor agonist, alters flash-evoked potentials in rats.* PHARMACOL BIOCHEM BEHAV **58**(4) 923–932, 1997.—This study examined the effects of the $GABA_A$ agonist THIP on flash-evoked potentials (FEPs) recorded from the primary visual cortex (VC) and superior colliculus (SC) of chronically implanted hooded rats. Animals were given IP injections of saline, and of 8, 16, and 24 mg THIP/kg body weight on separate days. Evoked potentials were recorded at 5, 20, and 35 min following injection. Animals were tested at a standard (22.5°C) room temperature. Most significant effects were observed at the 20- and 35-min recording intervals for both the 16 and 24 mg/kg doses, with effects at the 24 mg/kg dose the most pronounced. VC P1 amplitude remained unchanged, while N1 was reduced to such an extent that it became positive, ultimately blending into the rising phase of a positive component appearing between N1 and P2. This positive component had a latency of about 6 ms longer than N1, and became larger in amplitude than P1 at the 24 mg/kg dose. P2 amplitude was drastically reduced, becoming negative. In contrast, components N2 and P3 were augmented, while the amplitude of N3 was unchanged. In the SC, P1 was augmented while P3 was reduced in amplitude. A biphasic (increase/decrease) effect was observed in the N4 complex. In both the VC and SC, latencies of most components were increased, with the late components in the VC increased to the greatest extent. A mild hypothermia was observed at 16 and 24 mg/kg. The results suggest that the $GABA_A$ receptor plays an important role in the elaboration of the middle (N1–P2) components of FEPs recorded from the rat VC, and that GABAergic mechanisms can influence other components in the VC and SC as well. © 1997 Elsevier Science Inc.

THIP GABA Hypothermia Visual cortex Superior colliculus Flash-evoked potentials Visual-evoked potentials Rats

THE flash-evoked potential (FEP) is a complex electrical response of the brain that occurs immediately following the presentation of a brief flash of light. Individual components of this potential are representations of neural pathways that are activated during the photic stimulation (26). Both cortical and subcortical FEPs are easily recorded from laboratory animals. Such FEPs, recorded in particular from the visual cortex (VC), are often used in pharmacological and toxicological experiments to assess the functional integrity of the brain (10, 19), because they provide an integrated view of neural activity and sensory processing (51). In addition to modulation by exogenous chemical agents, alterations in FEP components can also reflect changes in internal states, such as body temperature or level of arousal (20,31,51). However, interpretation of any changes in FEPs is difficult because little is known about the neuronal processes responsible for peak generation. Increased knowledge of the neurotransmitters involved in the generation of individual components could, therefore, greatly increase the utility of FEPs.

In this regard, gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian visual system, and is believed to play a major role in information processing in the cerebral cortex (52). Prior work in cats has demonstrated that the first negative peak (N1) of the VC EP is enhanced, while the subsequent positivity (P2) is decreased in amplitude by those agents that decrease GABAergic inhibition [e.g., bicuculline; (54)].

Past investigations of the effects of GABAergic drugs on the rat VC FEP have concentrated on the late slow negative wave (N3) and subsequent afterdischarge (AD), usually leaving primary and secondary components without analysis. Furthermore, in these studies the results have often been conflicting/confus-

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ing. For example, King (35) reported that administration of both GABA antagonists (bicuculline, picrotoxin, and pentylenetetrazol) and GABA agonists (muscimol and imidazole acetate) produced dose-dependent increases in the AD. Another study (42) demonstrated that gamma-vinyl-GABA increased N3 and the AD while sodium valproate decreased N3 and the AD, even though both compounds inhibit GABA transaminase, thereby increasing brain GABA levels. Finally, Shearer et al. (48) reported that dipropylacetic acid (DPA) attenuated P3–N3 and the AD but had no effect on the early components of the VC FEP. Because DPA administration increases GABA concentrations in the cerebral cortex (27), they concluded that GABA probably plays no role in the elaboration of these early components.

GABA is unevenly distributed in the brain, with the superior colliculus (SC) being one of the areas with the highest concentration (44). Enhancing collicular GABAergic systems (e.g., via microinjection of muscimol) results in a reduction of behavior, while microinjections of picrotoxin or bicuculline produce behavioral patterns resembling either approach or defensive movements, depending on the site of injection (17). The prominent presence of GABA in this structure suggests that it should also play an important role in the generation of electrical activity recorded there, but this has yet to be investigated via the rat FEP.

Like many other neurotransmitter systems, there have now been identified pharmacologically and functionally distinct GABA receptor subtypes: $GABA_A$, $GABA_B$, and $GABA_C$ (13). The $GABA_A$ receptor directly controls a Cl^- ionophore, and has interacting binding sites for benzodiazepines, barbiturates, neurosteroids, picrotoxin, and ethanol (13,45). 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol (THIP) is a potent, direct $GABA_A$ agonist that is structurally related to muscimol, and easily penetrates the blood–brain barrier (36). Although used to investigate a number of presumed GABAergic-related behaviors [e.g., ethanol intake; (14)], it has yet to be used in conjunction with FEPs.

The present study employed THIP in an effort to assess the involvement of the $GABA_A$ receptor system in the production of the primary and secondary components of the FEP recorded from both the VC and SC of hooded rats. The results demonstrate that direct activation of $GABA_A$ receptors alters FEPs recorded from both structures, but in a very component-selective manner.

METHOD

Subjects and Surgical Preparation

Adult male Long–Evans hooded rats, weighing 350–485 g at the time of surgery, were tested. At least 2 weeks before testing, recording electrodes in the SC and/or VC were implanted under pentobarbital anesthesia. SC recordings were made from a twisted pair of nichrome wires, insulated to the tip, with an intertip distance of 1 mm. With the head in a horizontal position, the bipolar SC electrode was implanted 6.5 mm posterior to bregma, 1.5 mm lateral to the left of the midline, and lowered 4.7 mm below the surface of the skull. The visual cortex electrode (0–80 × 1/8" stainless steel screw) was placed 7 mm posterior to bregma and 3 mm lateral to the right of the midline. Similar screw electrodes placed over the ipsilateral and contralateral frontal cortex provided for a monopolar recording reference and grounding, respectively. Some of the animals also had a VC electrode implanted in the left VC. All electrodes were led to an Amphenol connector, and the whole assembly was secured to the skull with additional screws and dental acrylic.

At the conclusion of the experiment, placements of the SC electrodes were histologically verified (32). Results for the SC are reported for those animals in which the lower member of the electrode pair penetrated the superficial layer of the superior colliculus (22). Specifically, data were obtained from the SC in 10 animals, both the SC and VC in 4 animals, and only the right VC in 6 rats with bilateral VC electrodes.

Recording Procedure

Electrophysiological recording utilized Microdot low noise cables. Evoked potentials were amplified with Tektronix 122 preamplifiers with high and low filter settings of 1.0 kHz and 0.8 Hz for the SC, and 250 and 0.8 Hz for the VC, respectively. Amplified waveforms were averaged (n = 100) by a laboratory computer (2000 Hz sample rate, 400 ms epoch). Fifty milliseconds of the epoch occurred prior to the application of the evoking stimulus. Evoking stimuli were presented with an interstimulus interval of 2 s. Data collection was controlled with a Modular Instruments, Inc., Signal Averaging Program. Animals were tested at a standard (22.5°C) room temperature.

Recordings were obtained while animals were located inside a shielded recording chamber with dim background illumination of about 3 lx. The testing box located inside the shielded chamber measured 5 × 10 × 10 inches, and was constructed of white Plexiglas on three sides, the top, and bottom, while the front panel was clear. Flash stimuli were presented by a Grass Model PS22C photostimulator with an intensity setting of 8. The flash lamp of the photostimulator was placed in a small sound-attenuating chamber to eliminate the auditory click present with each flash. The flash lamp was positioned outside of the shielded recording chamber, and was visible to the rat through a clear Plexiglas window.

The animals were given 2 successive days of familiarization to the testing procedures in which the evoking stimuli were presented. This adaptation was followed by 3–6 days, prior to actual data collection. On each testing day, 30 min prior to testing animals received one drop of 1% opthalmic atropine in each eye, to maintain constant pupil dilation. Five minutes prior to testing, the animals were injected intraperitoneally on separate days with physiological saline (0.9% sodium chloride, 2 ml/kg), 8, 16, or 24 mg THIP/kg body weight. The sequence of injections was counterbalanced. Animals were given 3–7 days between tests. Evoked potentials were collected at 5, 20, and 35 min following injection. Immediately after testing, the animal was placed in a restraining tube, and a rectal thermistor probe (YSI No. 402) was inserted 10 cm into the rectum. A rectal temperature reading (YSI 44TA Tele-Thermometer) was then taken.

THIP dose selection was based largely on prior research, and was designed to provide a behaviorally relevant range of doses. Thus, a 16 mg/kg dose of THIP significantly increases voluntary ethanol intake in rats (14), but also significantly reduces locomotion (1). A dose of 8 mg/kg has no effect on locomotion (1), but would be expected to reduce grooming (6), while a 24 mg/kg dose is near the level of complete behavioral suppression (28). Postinjection test intervals facilitated the tracking of THIP-induced changes in individual FEP components, as noted in pilot observations.

Measurement and Statistical Analysis

For the VC FEP data (n = 10), baseline-to-peak amplitudes and peak latencies were obtained for seven components (P1, N1, Pn, P2, N2, P3, and N3). Component Pn was a small inflection on the rising phase of component N1, occurring between the N1 and P2 peaks, during baseline conditions (see

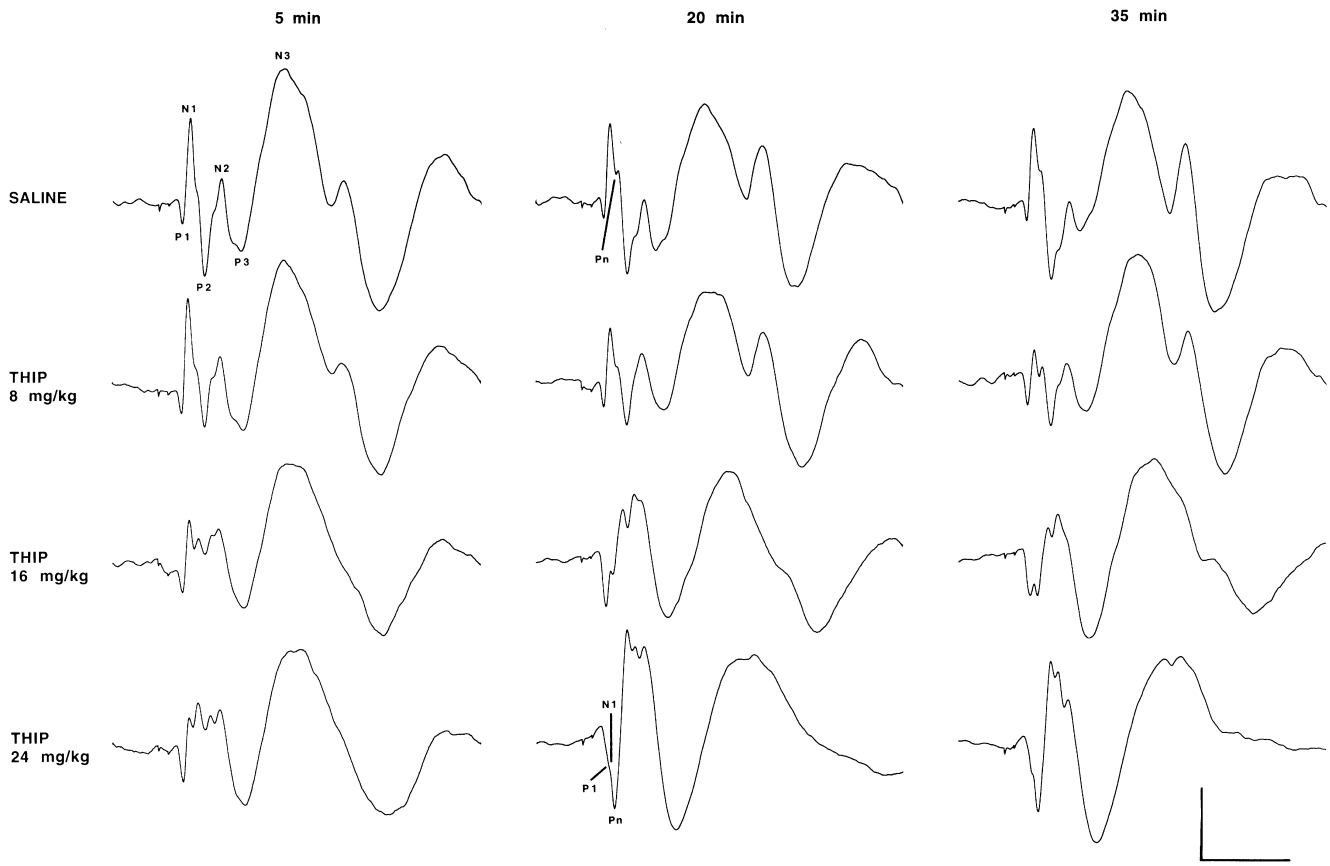

FIG. 1. Group average VC FEP waveforms ($n = 10$) for each dose of THIP and each time interval. Calibration: 50 μvolts and 100 ms.

Fig. 1). Components P1, P3, and N4 were similarly analyzed in the SC waveforms ($n = 14$). However, the N4 component was subdivided into N4a, N4b, and N4c (see Fig. 4), on the basis of previous work indicating the possible emergence of a pronounced positive deflection in the middle of N4 following some drug treatments [e.g., (30)]. The baseline-to-peak amplitudes consisted of the difference between the mean voltage of 50 ms of prestimulus activity and the peak voltage. Peak latencies were measured from the onset of the evoking stimulus.

Data were subjected to two factor (i.e., THIP dose and time) repeated measures analyses of variance. When a significant main effect, or drug × time interaction, was found, individual means were compared with Dunnett's test. The saline treatment and 5-min recording interval data served as the basis for comparisons in the Dunnett's tests. In all of the analyses, statistical significance was assumed when $p < 0.05$ for two-tailed comparisons. Changes in evoked potential amplitudes and latencies resulting from time-related factors are not included. Likewise, significant drug × time interactions are described only in relation to the main effects of the drug.

RESULTS

Visual Cortex

Amplitude. Group mean evoked potentials are displayed in Fig. 1, while the amplitude data are presented in Fig. 2. THIP produced profound changes in components N1, Pn, and N2 of the VC evoked potential, increased the amplitude of components N2 and P3, but had no significant effect on components P1 and N3: P1, $F(3, 27) = 2.40$, $0.1 > p > 0.05$; N1, $F(3, 27) = 10.392$, $p < 0.001$; Pn, $F(3, 27) = 7.222$, $p < 0.005$; P2, $F(3, 27) = 11.267$, $p < 0.001$; N2, $F(3, 27) = 9.592$, $p < 0.001$; P3, $F(3, 27) = 7.376$, $p < 0.001$; N3, $F(3, 27) = 1.120$, $p > 0.1$. Significant drug × time interactions were also present for the middle components: Pn, $F(6, 54) = 3.340$, $p < 0.01$; P2, $F(6, 54) = 3.521$, $p < 0.005$; N2, $F(6, 54) = 4.062$, $p < 0.005$; P3, $F(6, 54) = 2.571$, $p < 0.05$.

In comparison to saline, no dose of THIP significantly altered the amplitude of either component P1 or N3. However, N1 was significantly reduced in amplitude by both the 16 and 24 mg/kg doses of THIP. In fact, N1 was reduced to such an extent that it was positive at both the 20- and 35-min recording intervals for both of these doses. Similarly, component Pn (which may or may not be present in the saline condition, but if it is, consists of a small inflection on the rising phase between N1 and P2) was increased to such an extent at both the 20- and 35-min recording intervals for both the 16 and 24 mg/kg THIP doses that it even exceeded the amplitude of component P1 with the 24 mg/kg dose. As shown in Fig. 1, component N1 in this condition became an inflection point between components P1 and Pn. The effects of THIP on component P2 resulted in almost a mirror-image of the influence on N1. P2 was significantly reduced in amplitude at the 5-min interval following administration of all doses of THIP, and at the 20- and 35-min intervals with both the 16 and 24 mg/kg doses. With these higher doses, P2 became negative.

Both components N2 and P3 were significantly increased in amplitude by both the 16 and 24 mg/kg doses of THIP. The

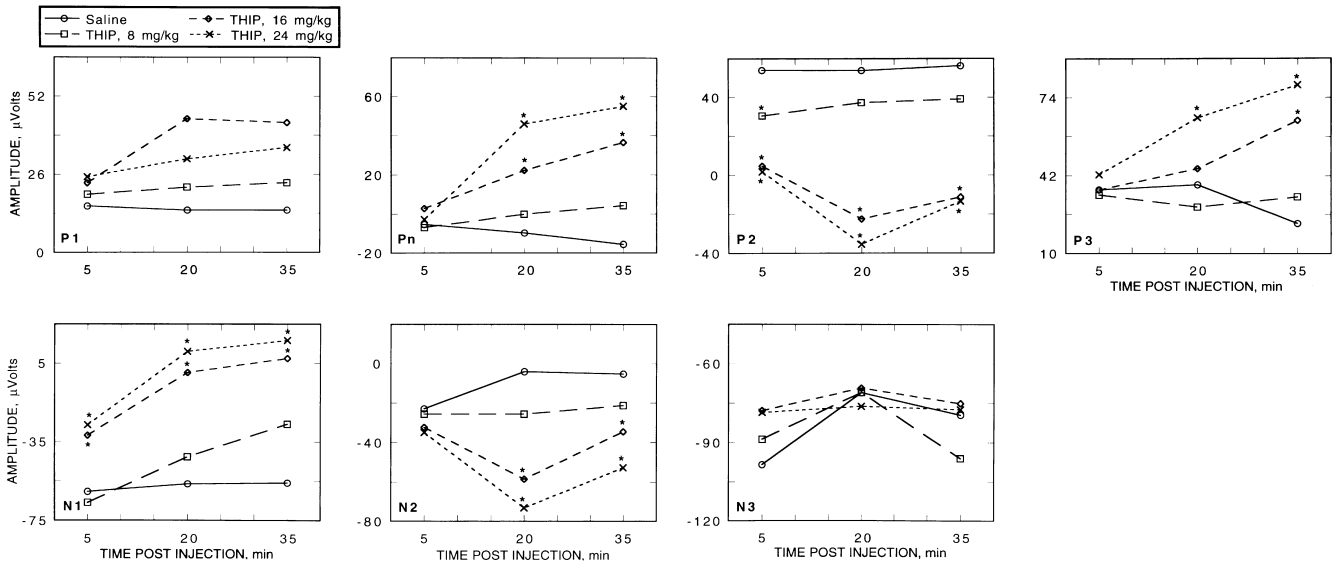

FIG. 2. Peak amplitudes of VC FEP components as a function of THIP dosage and time interval. Component notation as in Fig. 1. *$p < 0.05$ in comparison to saline.

augmentation occurred at the 20- and 35-min recording intervals in all cases, except the 16 mg/kg dose did not significantly alter component P3 at the 20 min interval.

Latency

The VC latency data are displayed in Fig. 3. THIP significantly altered the latency of most of the components: P1, $F(3, 27) = 17.002$, $p < 0.001$; N1, $F(3, 27) = 1.166$, $p > 0.10$; Pn, $F(3, 27) = 2.097$, $p > 0.10$; P2, $F(3, 27) = 2.980$, $p < 0.05$; N2, $F(3, 27) = 4.001$, $p < 0.025$; P3, $F(3, 27) = 12.078$, $p < 0.001$; N3, $F(3, 27) = 11.145$, $p < 0.001$, although the influence was not uniform across components or across time, the latter being reflected in significant drug × time interactions: P1, $F(6, 54) = 3.721$, $p < 0.005$; Pn, $F(6, 54) = 3.034$, $p < 0.025$; P3, $F(6, 54) = 2.481$, $p < 0.05$; N3, $F(6, 54) = 4.859$, $p < 0.001$).

Both the 16 and 24 mg/kg doses of THIP significantly increased the latency of components P1, P3, and N3 at both the 20- and 35-min recording intervals, with the exception of P3, which was not altered at the 35-min interval by the 16 mg/kg dose. In addition, N3 was significantly increased in latency at the 5-min interval by the 24 mg/kg dose. Component P2 was significantly increased in latency by only the 24 mg/kg dose of THIP. Components Pn and N2, on the other hand, were de-

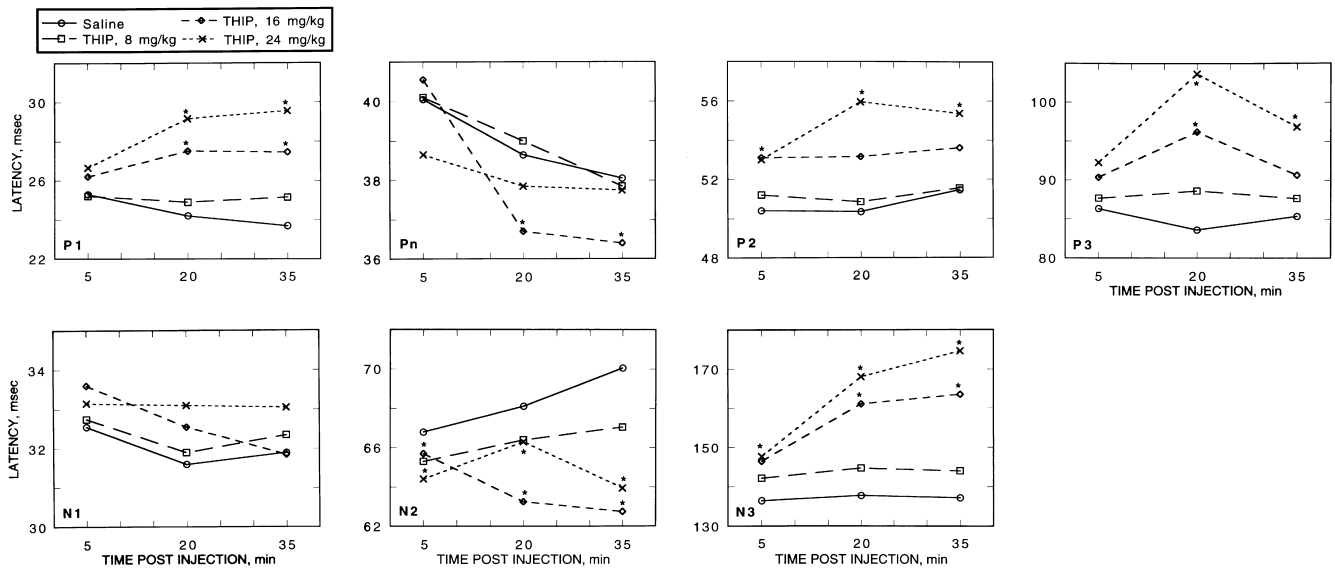

FIG. 3. Peak latencies of VC FEP components as a function of THIP dosage and time interval. Component notation as in Fig. 1. *$p < 0.05$ in comparison to saline.

creased in latency by THIP. For N2, the decrease occurred at all time intervals following administration of both the 16 and 24 mg/kg doses, while Pn was decreased in latency at the 20- and 35-min intervals by only the 16 mg/kg dose of THIP. Finally, component N1 was not significantly altered by any dose of THIP in comparison to the saline latencies.

Superior Colliculus

Amplitude. Figure 4 contains group average potentials for the SC, while the amplitude data are shown in Fig. 5. Compared to THIP-induced changes in the VC, the results in the SC are not as dramatic. Nonetheless, all components except N4c were significantly altered in amplitude by THIP: P1, $F(3, 39) = 17.480$, $p < 0.001$; P3, $F(3, 39) = 9.332$, $p < 0.001$; N4a, $F(3, 39) = 29.816$, $p < 0.001$; N4b, $F(3, 39) = 26.273$, $p < 0.001$; N4c, $F(3, 39) = 1.330$, $p > 0.10$. Significant drug × time interactions were present for these same components as well: P1, $F(6, 78) = 5.096$, $p < 0.001$; P3, $F(6, 78) = 2.301$, $p < 0.05$; N4a, $F(6, 78) = 5.223$, $p < 0.001$; N4b, $F(6, 78) = 6.457$, $p < 0.001$; N4c, $F(6, 78) = 1.195$, $p > 0.10$.

Component P1 was significantly increased in amplitude by both the 16 and 24 mg/kg doses of THIP at all time intervals, while P3 was reduced at all time intervals by both the 8 and 16 mg/kg doses, and at the 5-min interval by the 24 mg/kg dose. THIP produced biphasic effects on components N4a and N4b. N4a was increased in amplitude at all time intervals by the 8 mg/kg dose, but decreased by the 24 mg/kg dose. The 8 mg/kg dose increased the amplitude of component N4b, though only at the 35-min reading. N4b was significantly reduced in amplitude by the 16 mg/kg dose at 20 min, and at all time intervals by the 24 mg/kg dose.

Latency

The latencies of all SC components were increased to some extent by THIP, as shown in Fig. 6: P1, $F(3, 39) = 7.716$, $p < 0.001$; P3, $F(3, 39) = 1.110$, $p > 0.10$; N4a, $F(3, 39) = 2.984$, $p < 0.05$; N4b, $F(3, 39) = 8.616$, $p < 0.001$; N4c, $F(3, 39) = 8.320$, $p < 0.001$, although different components were influenced by different doses and at different time intervals, as indicated by significant drug × time interactions: P1, $F(6, 78) = 2.985$, $p < 0.025$; P3, $F(6, 78) = 2.671$, $p < 0.025$; N4a, $F(6, 78) = 2.315$, $p < 0.05$.

The latency of P1 was significantly increased at 20 and 35 min by the 16 mg/kg dose, and at all time intervals by the 24 mg/kg dose. In contrast, P3 was increased in latency at only the 5-min interval by all three doses of THIP. The 8 mg/kg dose of THIP increased the latency of N4a at both the 5- and 20-min intervals, while the 16 and 24 mg/kg doses increased

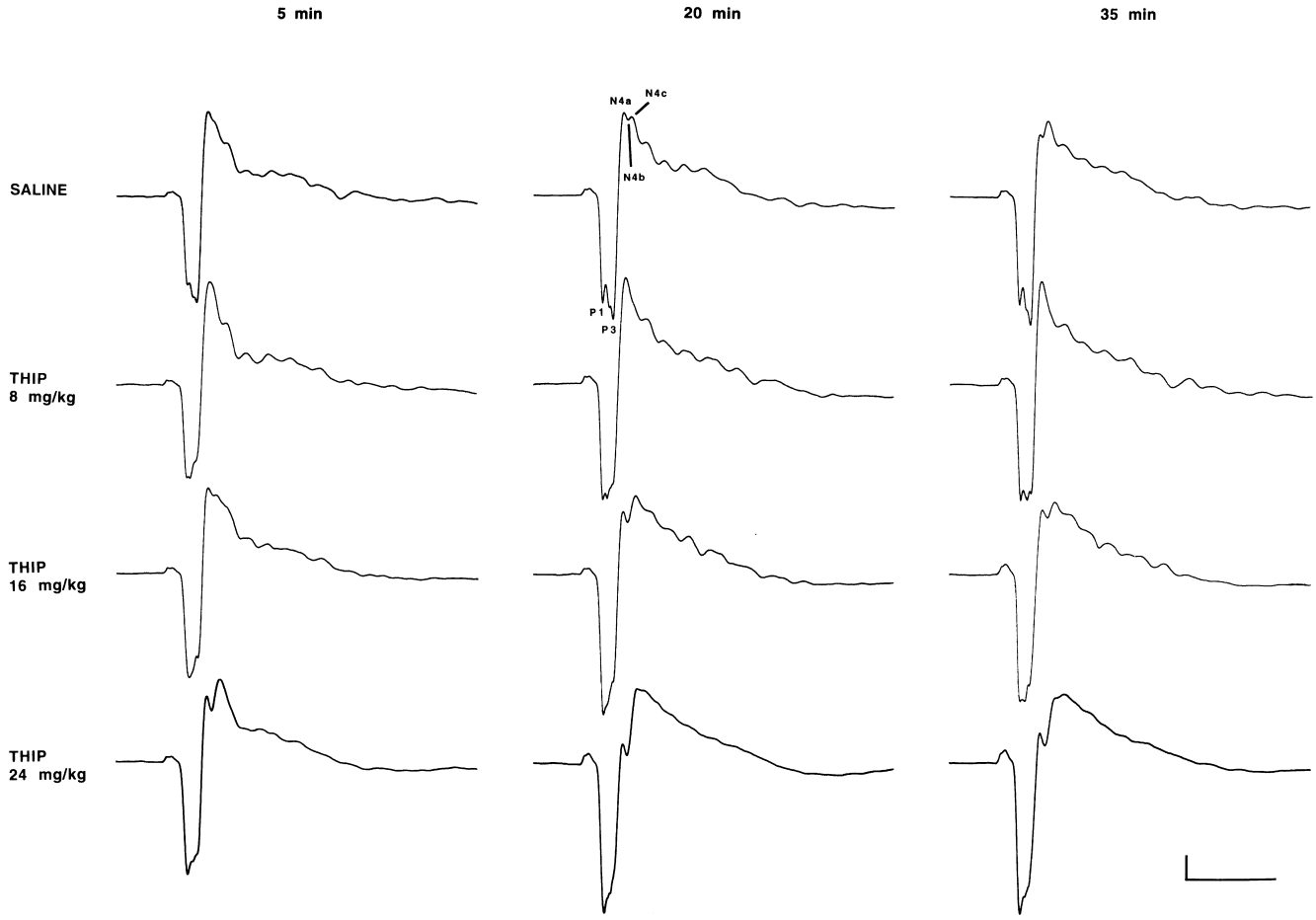

FIG. 4. Group average SC FEP waveforms ($n = 14$) for each dose of THIP and each time interval. Calibration: 50 μvolts and 100 ms.

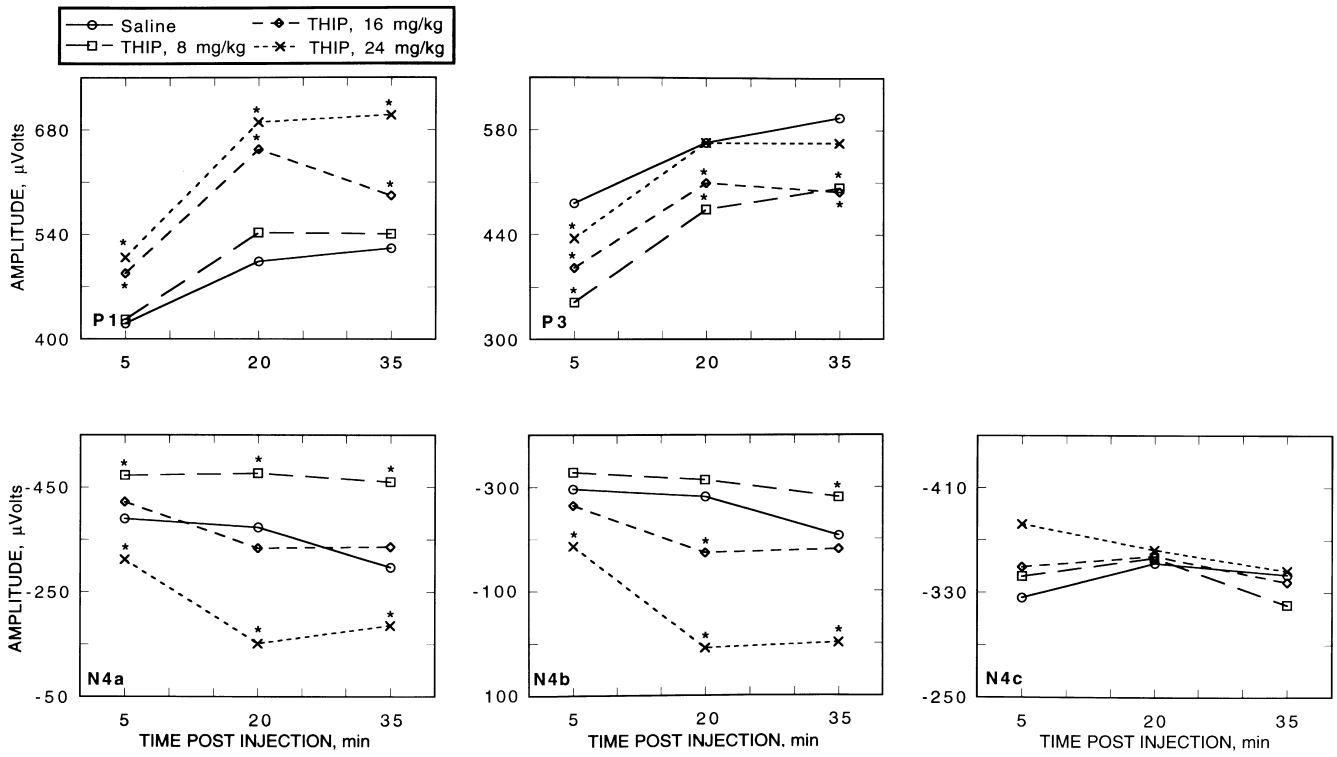

FIG. 5. Peak amplitudes of SC FEP components as a function of THIP dosage and time interval. Component notation as in Fig. 4. *$p < 0.05$ in comparison to saline.

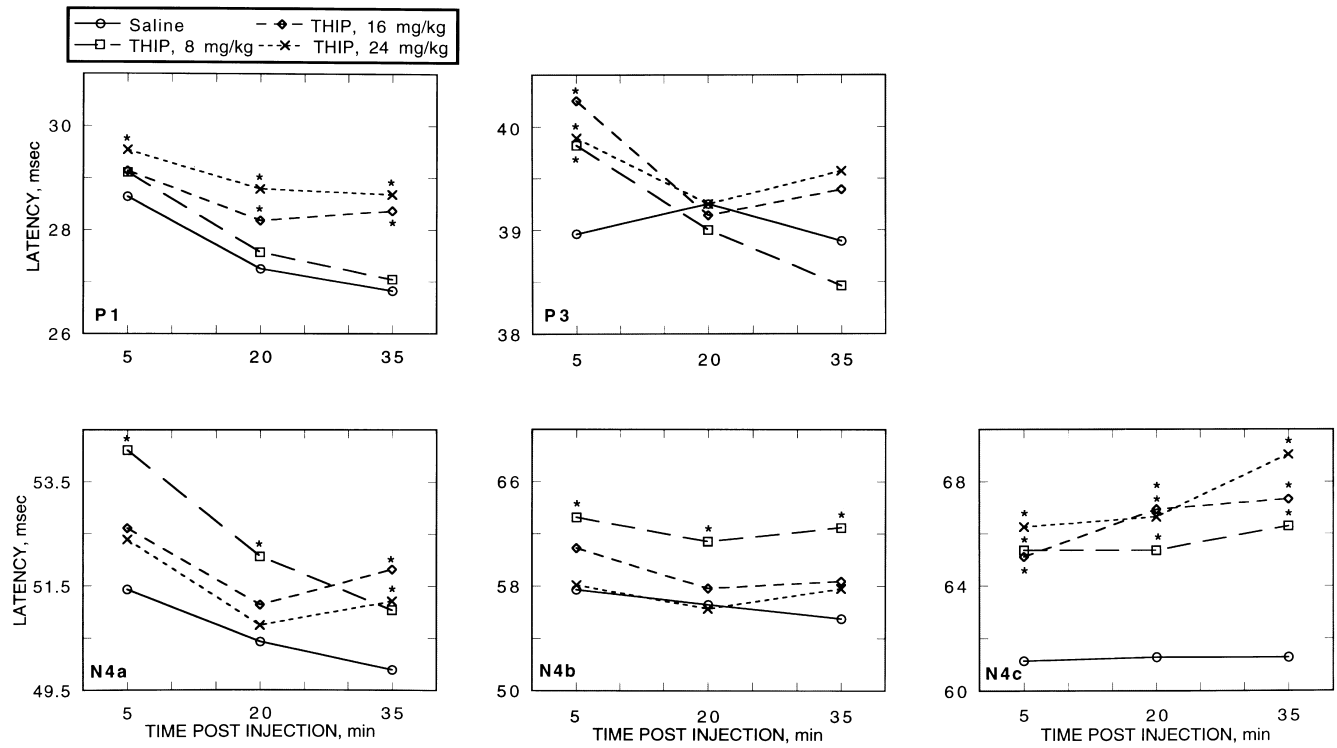

FIG. 6. Peak latencies of SC FEP components as a function of THIP dosage and time interval. Component notation as in Fig. 4. *$p < 0.05$ in comparison to saline.

the latency of this component at the 35-min interval. N4b was increased in latency by only the 8 mg/kg dose, while all doses increased the latency of component N4c.

Body Temperature

THIP produced a significant hypothermia at both the 16 and 24 mg/kg doses: $F(3, 39) = 22.183$, $p < 0.001$. The mean body temperature readings for 16 rats were as follows: Saline, 37.94°C ± 0.085 (SEM); 8 mg/kg, 37.79°C ± 0.081; 16 mg/kg, 37.28°C ± 0.124; 24 mg/kg, 36.94°C ± 0.163.

DISCUSSION

In the present study, THIP produced significant effects on FEPs recorded in both the VC and SC, mainly at the 16 and 24 mg/kg doses, with effects at the 24 mg/kg dose the most pronounced. In the VC, P1 amplitude remained unchanged, while N1 was reduced to such an extent that it became positive, ultimately blending into the rising phase of component Pn, the latter component becoming larger in amplitude than P1 at the 24 mg/kg dose. P2 amplitude was drastically reduced, becoming negative. In contrast, components N2 and P3 were augmented, while the amplitude of N3 was unchanged.

VC components N1 and P2 were most drastically altered in the present study by THIP, suggesting some sort of relationship between the two, independent of the preceding component P1. That N1 is relatively independent of P1 was demonstrated previously (23), as evidenced by a low correlation between component amplitudes. Also of interest, these authors determined the surface distribution of all the major peaks of the FEP recorded from the posterior cortex of rats, and found that the generators of peaks N1 and P2 were located more posterior than those of the other peaks.

Using cats, Zemon et al. (54) recorded VC EPs in response to luminance increment. They found that topical application of bicuculline greatly enhanced N1, eliminated P2, but had little effect on P1. On the basis of these results, the authors suggest that N1 represents an excitatory process, while P2 represents GABA-mediated inhibition. To the extent that similar neuronal processes are involved in the production of visual EPs in the cat and rat, it might therefore be expected that in the present study, P1 would remain unchanged, N1 would be greatly reduced, while P2 would be enhanced by THIP. This prediction is supported for components P1 and N1, but not P2.

In the adult rat visual cortex, GABA terminals are found in every layer, with the greatest density of synaptic contacts occurring in layer IV (7). However, these authors note that there is an interesting difference in the proportions of GABA contacts in the visual cortex of rodents (11–12%) in comparison to that of cats, macaques, and humans (16–17%). They speculate that this may reflect a smaller proportion of GABA neurons in the rat visual cortex (one in seven) than in the cat and monkey (one in five), as shown in prior studies (8,9). In addition, a higher number of overall synaptic contacts was found in the rat cortex, reflecting in part a greater number of contacts on dendritic spines in the rat than in the other species. Given that thalamocortical afferents end preferentially on dendritic spines of layer IV (7), the unusual pattern of GABA circuitry in the rat visual cortex could account for the differences in the effects of GABAergic drugs on VC EPs observed in the present study in comparison to effects found in the cat.

In the $GABA_A$ receptor, a chloride (Cl^-) ionophore is controlled by the GABA receptor, and can be modulated at other binding sites by other compounds, such as barbiturates. At low (subanesthetic) doses, barbiturates enhance the affinity of $GABA_A$ receptors for GABA, and also increase the GABA-induced mean Cl^- channel opening time. At anesthetic doses, barbiturates directly increase channel openings (45). In past work (33), we examined the effects of a range of subanesthetic to anesthetic doses (3–40 mg/kg) of pentobarbital on VC FEPs in rats. In contrast to the effects of THIP in the present study, pentobarbital enhanced the amplitude of both components P1 and N1, produced biphasic effects on P2 and N2 (enhancement at midrange doses, with return to baseline at 40 mg/kg), and depressed P3 and N3, even at low doses. Thus, compounds working on the same receptor complex can produce drastically different effects on EPs, illustrating the complexities of the circuits involved in component production.

The dissimilarity between the effects of THIP and pentobarbital on VC FEPs in some respects resembles the results of animal discrimination studies. In rats trained to discriminate pentobarbital from saline, THIP yields only intermediate levels (40–60%) of pentobarbital-lever responding (28). Also, in rats trained to discriminate THIP from the no-drug condition, pentobarbital does not substitute for THIP (5).

GABA is formed from glutamic acid by the enzyme glutamic acid decarboxylase (GAD), while GABA-transaminase (GABA-T) metabolizes GABA to succinic semialdehyde. Compounds used in the past to manipulate GABA levels in the brain include gamma-vinyl GABA (GVG), an irreversible inhibitor of GABA-T, which causes a dose-dependent increase in brain GABA levels, and sodium valproate (VPA), which is thought to inhibit GABA-T, but also stimulates GAD, increasing GABA synthesis (29). Most past studies of the rat VC FEP employing such compounds have concentrated on component N3 and the subsequent AD, with mixed and often contradictory results.

In 1981, Myslobodsky and Morag (42) reported that GVG increased the amplitude of N3 and the AD, while VPA decreased N3 and the AD. Furthermore, VPA antagonized the GVG-induced enhancement. A similar study (40), found suppression of N3 and the AD by both VPA and diazepam. Diazepam-induced attenuation of the AD has also been reported by others (11). Benzodiazepines, such as diazepam, increase the affinity of $GABA_A$ receptors for GABA, and also increase the frequency of GABA-induced channel openings (45). However, these results on the effects of GABA-acting compounds on the AD are difficult to interpret, given the findings of a study by King (35). He observed that IP administration of both GABA antagonists (bicuculline, picrotoxin, and pentylenetetrazol) and GABA agonists (muscimol and imidazole acetate) produced dose-dependent increases in the VC AD.

Relatively few past studies have examined the effects of GABA-altering drugs on the primary components of the rat VC FEP. One study noted that single doses of the anticonvulsants trimethadone, dipropylacetic acid (DPA), and diphenylhydantoin failed to alter the peak-to-peak amplitude or peak latency of components P1 through P3, with the exception of a suppression of P3–N3 amplitude by trimethadione (47). Finally, Shearer et al. (48) reported that a single dose of DPA (200 mg/kg) attenuated P3–N3 amplitude and the AD, but had no effect on the amplitude or latency of any of the earlier components of the VC FEP. Because DPA administration increases GABA concentrations in the cerebral cortex (27), they concluded that GABA-mediated pathways probably have no direct influence on these early components. The results of the present study, employing the $GABA_A$ agonist THIP, suggest that these earlier studies employed pharmacological

treatments that were not specific enough to allow an adequate assessment of the role of GABA in component production.

Given the rather dramatic changes in FEPs produced by THIP, one might expect changes in behavior as well. This was indeed the case, and occurred in a dose-dependent manner. Thus, while casual observations did not detect any obvious behavioral changes at the 8 mg/kg dose of THIP, over half of the rats appeared relaxed and less active than normal at the 16 mg/kg dose, and the animals did not move much at all at the 24 mg/kg dose. This is generally in line with past research, although more systematic observations in some studies demonstrate that the estimated ED_{50} to selectively suppress grooming behavior in the open field is 1.0 mg/kg (6), while locomotion is significantly reduced at 16 mg/kg IP (1). Other work shows that THIP (1–30 mg/kg) produces a dose-dependent decrease in the rate of lever pressing in rats, with complete suppression occurring at 30 mg/kg (28).

The general dose-dependent decrease in behavior is of interest in regard to the lack of effect of THIP on the amplitude of VC component N3. Current research indicates that in the normal state of the animal, the amplitude of N3 reflects sensitization to the evoking stimulus, and that repeated testing is necessary to maximize the amplitude of this component (21). However, motor activity is known to reduce the amplitude of component N3 (25), and the results of past pharmacological studies measuring the amplitude of this component have often been interpreted in terms of level of arousal, such that N3 is reduced in amplitude by those treatments that either increase or decrease arousal level [e.g., (20)]. Thus, both alcohol (32) and pentobarbital (33) reliably reduce the amplitude of this component.

In marked contrast to these two central nervous system depressants, however, the anesthetic urethane (ethyl carbamate) fails to suppress N3. Surprisingly in this regard, when Dyer and Rigdon (24) investigated the influence of urethane (0.25–1 g/kg) upon VC FEPs in hooded rats, in some respects their results were strikingly similar to those observed in the present study. In particular, peak N1 disappeared at the 0.5–1 g/kg dosages, while P3 was augmented and N3 amplitude remained unaffected (P1 was not examined). Although effects on other components were dissimilar (P2 was increased and N2 decreased in amplitude), the elimination of N1 combined with a lack of influence on N3 argues for some common underlying neuropharmacological modifications produced by urethane and THIP.

The mechanism(s) by which urethane produces anesthesia are unknown. Because urethane has anticonvulsant properties (15), GABA receptor involvement might be suspected. However, mixed results were found by Shirasaka and Wasterlain (49) when they examined the effect of urethane on evoked potentials recorded in the dentate gyrus produced by ipsilateral perforant path stimulation; they found that urethane decreased short interstimulus interval-dependent paired-pulse inhibition. Because such paired-pulse inhibition is mainly $GABA_A$ mediated (2), this finding indicates that urethane does not act as a $GABA_A$ agonist. On the other hand, urethane also suppressed the population spike amplitude in input/output response examinations, a finding similar to that produced by known $GABA_A$ receptor agonists (3).

Still other studies suggest a link between urethane and excitatory amino acid (EAA) neurotransmitters [e.g., (16,41). The role of excitatory amino acids in the production of the rat VC FEP was recently investigated by Siegel and Sisson (50), who also found results similar to those of Dyer and Rigdon (24). Using a cortical cup allowing perfusion over the cortical surface, they recorded FEPs during perfusion with kynurenic acid, a nonselective blocker of EAA receptor sites. Components N1 and N2 were abolished in a dose-dependent manner, while P1 and P2 were unaffected (P3 and N3 were not examined). The authors interpreted their data as follows: P1 is the correlate of the presynaptic geniculate volley, N1 represents a geniculocortical synaptic process produced by EAA release, P2 represents a nongeniculate, non-EAA mediated postsynaptic potential, while N2 reflects an EAA-mediated intracortical or subcortical (but not direct dorsal lateral geniculate) input to the VC. These conclusions do not preclude the important involvement of GABA in the elucidation of these components, however, since although the visual pathways use excitatory neurotransmitters for transmitting information, at each excitatory synapse local GABA-releasing cells gate and modify the responses produced in the postsynaptic cells (52).

FEPs recorded from the SC were also studied in the present experiment. In addition to intrinsic GABAergic neurons in the SC (38), there is also an apparent GABAergic projection from the substantia nigra pars reticulata to the intermediate layers of the SC (4,53), as well as a possible GABAergic input from the substantia nigra pars lateralis to the superficial layers of the SC (46). Studies in rodents indicate that the superior colliculus is involved in the initiation of a number of physiological and behavioral reactions to suddenly appearing stimuli (18). Enhancing collicular GABAergic systems (e.g., via microinjection of muscimol) results in a reduction in behavior, while microinjections of picrotoxin or bicuculline produce behavioral patterns resembling either approach or defensive movements, depending on the site of injection (17).

Within the SC, the highest level of GABA is found in the superficial gray layer (43). Likewise, the density of both $GABA_A$ and $GABA_B$ receptor subtypes is highest in the superficial gray layer, although there is a greater density of $GABA_B$ receptors than $GABA_A$ receptors in the rat SC (39). Postsynaptic potentials recorded from the superficial gray layer in SC slices following stimulation of the optic layer can be altered by the application of GABAergic compounds. Specifically, both GABA and muscimol produce dose-dependent biphasic enhancement and depression of the postsynaptic potentials, while (−)-baclofen (an agonist for $GABA_B$ receptors) has only an inhibitory effect (43). Thus, both $GABA_A$ and $GABA_B$ receptors are involved in the electrophysiology of the SC.

In the present study, THIP augmented the amplitude of SC FEP component P1, reduced P3, and altered N4a and N4b in a biphasic (increase/decrease) manner. In past work, pentobarbital (33) produced very similar, though more pronounced changes in these same SC components. Thus, 18–40 mg/kg pentobarbital augmented the amplitude of component P1, while the same dosages reduced or eliminated P3, and resulted in a biphasic effect on N4. It, therefore, appears that $GABA_A$ receptors are involved in the elaboration of the FEP recorded from the SC. However, given the relatively small magnitude of the effects of THIP on FEPs recorded from the SC, other GABA receptor subtypes and/or other neurotransmitters probably play an even greater role.

Changes in component latency were found in both the VC and SC in the present study, as well as a mild THIP-induced hypothermia. However, the hypothermia observed was not sufficient to account for the changes in component latencies. For example, for component P1, the estimated latency increase from a 1.0°C decrease in body temperature is about 1.4–1.7 ms (31). Component P1 was increased by about 5–6 ms by the 24 mg/kg dose of THIP, even though there was a drop in body temperature of only about 1.0°C. Furthermore,

some of the components decreased in latency, which would not be accounted for by drug-induced hypothermia.

In conclusion, the results of the present study demonstrate that THIP produces significant changes in the configuration of FEPs. However, the neurogenesis of both cortically and subcortically recorded FEPs involves complex circuits that presumably contain a variety of neurotransmitters. While activation of $GABA_A$ receptors will modify the FEP, it is not known if this is a primary or secondary effect, because the GABA system interacts extensively with other neurotransmitter systems, such as acetylcholine, norepinephrine and serotonin [e.g., (37)]. Because these other systems also influence FEPs [e.g., (12,34)], GABA may play a role in modulating these other transmitters rather than a primary role in component production.

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