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Taurine Action on Mitral Cell Activity in the Frog Olfactory Bulb *In Vivo*

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Abstract

Taurine (TAU) is a free amino acid that is particularly abundant in the olfactory bulb. In the frog, TAU is located in the terminations of the primary olfactory axons and in the granular cell layer. TAU action seems to be associated with gamma amino butyric acid (GABA), the main inhibitory neurotransmitter involved in the processing of the sensory signal. The present study was designed to assess the action of TAU *in vivo* during the olfactory network's stimulation by odors. It was performed by recording the single-unit activity of mitral cells, the main bulbar output neurons. TAU effects were tested on both their spontaneous and odor-induced firing activity. Interactions between TAU and GABA were examined by analyzing TAU effects under the selective blocking action of $GABA_A$ or $GABA_B$ antagonists. TAU was found to suppress the spontaneous firing of mitral cells, mainly without altering their odor response properties. By testing GABA antagonists, we further show that TAU action is associated with GABAergic inhibitory mechanisms mainly via $GABA_B$ receptors. Thus, TAU action clearly reduces background activity in favor of the emergence of the odor-induced activity in the same manner as GABA action does via $GABA_B$ receptors. As a conclusion, we propose that, in the frog olfactory bulb, the joint actions of TAU and GABA may favor the processing of the primary sensory information by increasing the signal to noise ratio.

Key words: frog, GABA, $GABA_B$ and $GABA_A$ receptors, odor coding, olfactory bulb, taurine

Introduction

Taurine (TAU; 2-aminoethanesulfonic acid), is, after glutamate, the most abundant free amino acid in the cerebral cortex and cerebellum (Kuriyama *et al.*, 1983; Pow *et al.*, 2002) and even more so in the olfactory bulb (OB) (Collins, 1974; Shimada *et al.*, 1984; Ashihara *et al.*, 1992; Brittebo and Eriksson, 1995; Ross *et al.*, 1995; Pow *et al.*, 2002). In the rat OB, TAU-like immunoreactivity was observed in periglomerular neurons, co-localized with tyrosine hydroxylase (Sakai *et al.*, 1987). In the frog OB, a recent microscopic study of TAU-like immunoreactivity reported TAU immuno-labeled primary fibers entering the OB and making densely visible glomerular neuropiles (Kratskin *et al.*, 2000). In this study, TAU was found in the granular cell layer through immunolabeled processes and cell bodies, the latter cells being hypothesized as being short axon cells co-localizing GABA and TAU.

From a functional point of view, TAU is still intriguing, and the question of whether TAU acts as a neurotransmitter or a neuromodulator in the central nervous system is still under debate today (Salceda and Pasantes-Morales, 1982; Haroutounian and Petrosian, 1998). Several lines of evidence suggest that TAU may act as an inhibitory neurotransmitter (Frederickson *et al.*, 1978; Wang *et al.*, 1998). However, although some specific molecular receptors to this amino acid have been identified (Lopez-Colome, 1981; Wu *et al.*, 1992), the molecular mechanisms underlying TAU inhibitory action on cell membranes remain mostly unknown. Taken together, data strongly suggest that TAU may act as a trigger for the release of various neurotransmitter in the brain, thus modulating neuronal activity rather than as a neurotransmitter in itself (Kuriyama *et al.*, 1978; Muramatsu *et al.*, 1978; Kontro *et al.*, 1984).

The first study where TAU and the OB were associated functionally was done by Kamisaki *et al.* (1996). It described the effects of TAU on gamma amino butyric acid (GABA) release from synaptosomes of the rat OB. TAU was found to play an important modulator role in regulating the depolarization-evoked GABA release via $GABA_B$ receptors. However, the impact of this approach is limited since data were obtained from synaptosomes and cannot be easily placed within the physiological context of the bulbar network. Further insights into the role of TAU in the OB were provided by a recent work in the rat *in vitro* (Puopolo *et al.*, 1998). Mitral cells were shown to be reversibly inhibited by application of TAU, the inhibition being antagonized by a $GABA_A$ antagonist but not by a $GABA_B$

antagonist. TAU inhibition was demonstrated to be exerted directly on mitral cells since it was maintained even when synaptic transmission was pharmacologically blocked. Taken together, the different authors who studied TAU action in the frog OB agree in closely associating TAU and GABAergic inhibitory mechanisms.

GABA has been previously demonstrated to be strongly involved in the integration of the odor signal performed by mitral cells via both $GABA_A$ and $GABA_B$ receptors in frogs *in vivo* (Duchamp-Viret and Duchamp, 1993, 1997; Duchamp-Viret *et al.*, 1993, 2000). More precisely, GABA was shown to act at the glomerular level via $GABA_A$ receptors on the mitral cell sensitivity to primary inputs by limiting their depolarization level and adjusting their response threshold (Duchamp-Viret *et al.*, 1993). GABA was also shown to modulate the signal to noise ratio of OB inputs via $GABA_B$ receptors since it suppressed the mitral cell spontaneous activity without affecting their responsivity to odors (Duchamp-Viret *et al.*, 2000).

The abundance of TAU in primary olfactory axons within glomeruli raises the hypothesis that TAU might be co-released with the primary excitatory neurotransmitter, namely glutamate. In the glomerular neuropile, the tight packaging of the primary olfactory fibers with the dendrites of the mitral and periglomerular cells might favor a TAU neuromodulation of GABAergic synaptic or extra-synaptic actions. Such a hypothesis is perfectly coherent with the numerous lines of evidence which show that TAU would act in a close association with GABA, chloride conductances and inhibitory actions in other brain structures (Lindquist *et al.*, 1983; Billard and Batini, 1991; Galarreta *et al.*, 1996; Wang *et al.*, 1998; Del Olmo *et al.*, 2000; McCool and Botting, 2000; Chepkova *et al.*, 2002). Until now, TAU action in the OB has never been studied *in vivo* during olfactory stimulation. TAU action in the frog OB is closely associated with GABAergic mechanisms (Kamisaki *et al.*, 1996; Puopolo *et al.*, 1998). This led us to deepen our understanding on the role of TAU on the OB network by studying TAU action on the two types of GABAergic inhibitory mechanisms that we previously studied in the same experimental conditions (Duchamp-Viret and Duchamp, 1993; Duchamp-Viret *et al.*, 1993, 2000). Given data obtained in the OB by Puopolo and coworkers (Puopolo *et al.*, 1998) and by our own group (Duchamp-Viret *et al.*, 2000), we hypothesized that TAU may act via both $GABA_B$ and $GABA_A$ receptors either directly or by enhancing GABA action by itself. In the present electrophysiological study, TAU effects on both the spontaneous activity and the odor intensity coding properties of mitral cells are described. The mechanisms of TAU action are further investigated by blocking selectively $GABA_A$ or $GABA_B$ actions using picrotoxin and saclofen respectively.

Materials and methods

Animal preparation

Before surgery, frogs (*Rana ridibunda*, $n = 43$) were locally anaesthetized by xylocaine (lidocaine 2%) application to the dorsal surface of the head. When animals failed to react to a pinprick of the skin, they were immobilized by a subcutaneous injection of 0.1 ml of D-tubocurarine (0.2%) and wrapped in wet gauze to preserve their respiration through the skin. At regular time intervals, xylocaine was reapplied to the wounds.

The cranial upper wall was resected to expose the OB and nerves. The meninges were then carefully dissected. The ipsilateral nasal cavity was prepared as previously described (Duchamp-Viret *et al.*, 1989), so as to stimulate the entire olfactory mucosa.

Stimuli

The odorants used (Merck, Lyon, France) were anisole, camphor, cineole, isoamyl acetate, limonene and methylamylketon. These compounds were previously found to be highly effective and clearly discriminated by mitral cells (for a review see Duchamp-Viret and Duchamp, 1997). Stimuli were delivered by a dynamic flow multistage olfactometer designed to ensure a precise control of the stimulation parameters as described elsewhere (Vigouroux *et al.*, 1988). Twenty different concentrations could be obtained from discrete dilutions of the saturated vapors at atmospheric pressure in the range from 1×10^{-6} to 5.62×10^{-2} of saturation. Stimuli were 2 s odor pulses delivered to the olfactory mucosa at a constant temperature (22°C) and flow rate (100 ml/min). During the stimulation procedure, a delay of at least 2 min elapsed between successive odor stimuli that were delivered from the lowest to the highest concentrations.

Electrophysiological recordings

Single-unit action potentials were recorded extracellularly by using metal-filled glass micropipettes (2 to 4 MΩ; Gesteland *et al.*, 1959), and electro-olfactograms (EOGs) were recorded with 50 µm diameter glass micropipettes filled with saline solution. The two signals were led online through conventional amplifiers to a Data Tape Recorder (Bio-Logic, Grenoble, France) and to a CED-1401 data acquisition system (Cambridge Electronic Design Ltd, Cambridge, UK) connected to a computer. Spike and EOG signals were filtered between 300 and 3000 Hz and between 0 and 30 Hz respectively. They were sampled at 15 kHz and 200 Hz respectively on the CED-1401 data acquisition system. The single unit nature of the recorded spikes was first controlled during the experiment by triggering the recorded cell near the background-noise on a storage oscilloscope. This allowed us to control the characteristics of the polyphasic spike of the cell studied in order to ensure that the same cell was recorded throughout all the experimental procedure. The single-unit activity was then triggered using the facilities

offered by the Spike2 language associated to the CED-1401 system. Spikes were detected using their waveform over a triggering level, and then by visual inspection of the consistency of the shape of the sorted spikes on the computer screen.

In the frog, mitral cells are dispersed in the external plexiform/mitral cell layer, which is oriented dorso-ventrally, at a depth of 300–1200 μm from the surface (Scalia, 1976; Scalia *et al.*, 1991). In previous experiments, using a collision test between a spontaneous orthodromic spike and an antidromic spike evoked by the electrical stimulation of the lateral olfactory tract, 90% of the cells recorded in this region were found to be mitral cells (Duchamp-Viret *et al.*, 1989).

Response determination

From the recorded data, the unit activity was discriminated using a window discriminator and the interspike intervals determined (Duchamp-Viret *et al.*, 1989). For each stimulation sequence, corresponding to one cell stimulated with one stimulus at one concentration, successive interspike intervals were counted for 30 s before and for 30 s after the stimulation (the 2 s odor delivery was included in the second 30 s period). Distributions of post-stimulus interspike intervals which were significantly different from the prestimulus distribution were identified using the Mann–Whitney *U*-test as described elsewhere (Duchamp-Viret *et al.*, 1989). The responses are presented as the average of the instantaneous frequency of each interspike interval (spikes/min).

Application of pharmacological agents

Depending on the experiments, the drugs used were taurine (10^{-3} M; Sigma, Lyon, France), picrotoxin (10^{-3} M; Sigma), a $GABA_A$ antagonist that blocks chloride channels, baclofen ($10^{-4}/10^{-3}$ M, RBI, Lyon, France), a specific agonist of $GABA_B$ receptors (Potapov, 1985; Potapov and Trepakov, 1986; Nickell *et al.*, 1994), or saclofen (10^{-4} or 10^{-3} M, RBI), a specific antagonist of $GABA_B$ receptors (Kabashima *et al.*, 1997). Drugs were diluted in amphibian Ringer adjusted to pH 7.4 (composition: 110.4 mM NaCl, 2.5 mM KCl, 2.4 mM $NaHCO_3$, 1.8 mM $CaCl_2$). During the experiment, 1–50 μl of the drug-containing solution was gently applied with a Hamilton syringe at the level of the olfactory nerves into the dissected cranial cavity. The cavity contains naturally ~50–150 μl of cerebrospinal liquid and can be experimentally rinsed by perfusing amphibian Ringer. This method of drug application has been used in several previous studies in the frog (Duchamp-Viret and Duchamp, 1993; Duchamp-Viret *et al.*, 1993, 1997, 2000)

Experimental paradigm

All cells were submitted to the following TAU protocol. Their spontaneous firing activity was first recorded at least 3 min before any drug application. Their responsiveness was then tested by delivering with a syringe a puff of each of the available odors at a concentration close to saturation at intervals of at least 1 min in order to determine their qualitative response profile. Using the olfactometer, cells were then submitted to increasing concentrations of one of the odors that elicited an excitatory response, in order to establish their concentration–response curves. At the end of this procedure, 1–5 μl of taurine (TAU) was carefully applied and the stimulation procedure with the syringe and/or the olfactometer was repeated.

To characterize the mechanisms of TAU action, some cells were further recorded after saclofen (SACLO-TAU protocol) and/or picrotoxin (PICRO-TAU protocol) pretreatment. In these cases, TAU was rinsed by repeated applications of amphibian Ringer into the opened cranial cavity until the cell returned to its spontaneous firing rate. Picrotoxin or saclofen was then applied 10–15 min prior to the second TAU application. When necessary, additional doses of TAU were applied in an attempt to counteract picrotoxin or saclofen action. Finally, in four cases, baclofen was applied when the successive applications of TAU did not counteract picrotoxin action.

Results

TAU action was tested on 52 mitral cells. Thirteen and 11 of these cells were also tested after picrotoxin and saclofen pretreatment respectively.

TAU action on mitral cell spontaneous activity

Tables 1 and 2 give cell mean spontaneous firing rates in the three experimental protocols and the percentages of cells with spontaneous firing partially or totally abolished by TAU alone and by TAU after picrotoxin and saclofen pretreatments.

As shown in these tables, TAU drastically alters mitral cell spontaneous firing activity. TAU alone (TAU protocol) decreases the spontaneous firing rate in 90% ($n = 47$) of the cells. This effect occurs on average within 30 ± 17s after drug

Table 1 Mitral cell spontaneous firing rates in control conditions, under TAU alone, and under TAU after picrotoxin (PICRO) or saclofen (SACLO) pretreatment

	TAU protocol ($n = 52$)		PICRO-TAU protocol ($n = 13$)		SACLO-TAU protocol ($n = 11$)	
	Control	TAU	PICRO	PICRO + TAU	SACLO	SACLO + TAU
Spontaneous firing rate (spikes/min)	27.6 ± 15.6	4.4 ± 7.9	69.9 ± 45.9	25.1 ± 18.5	27.6 ± 21.2	25.3 ± 25.5
Necessary doses to induce TAU effect	5–20 μl at 10^{-3} M		30–130 μl at 10^{-3} M		20–50 μl at 10^{-4} M	

Table 2 Percentages of affected cells and percentages of firing rate changes under TAU alone or after picrotoxin (PICRO) or saclofen (SACLO) pretreatment

TAU action on spontaneous firing activity	TAU protocol (n = 52)	PICRO-TAU protocol (n = 13)	SACLO-TAU protocol (n = 11)
No change (firing rate difference <10%)	10	31	73
Partial depression	8 (% of change: –70.9 ± 4.8)	46 (% of change : –53.7 ± 19.5)	0
Total spike disappearance	82	23	27

application. This decrease is divided into 82 % of total spike disappearance and 8% of strong firing decrease.

When TAU is preceded by an application of picrotoxin, which blocks chloride channels (PICRO-TAU protocol), the inhibitory effect of TAU drops to 69% (46 + 23%). It drops to 27% after saclofen pre-treatment (SACLO-TAU protocol). Thus, saclofen, and picrotoxin to a lesser extent, seem to be able to counteract TAU action.

TAU action under picrotoxin seems to be reduced in strength (Table 2) since it induces mainly partial firing decrease with respect to total spike disappearance. However, this reduction is relative since picrotoxin induced a strong initial spontaneous firing rate increase in the period preceding TAU application (Table 1). Under picrotoxin, several successive applications of TAU (2–4 doses; 30 to 150 µl; n = 9) were necessary to overcome the picrotoxin desinhibition and to inhibit the spontaneous firing. However, four cells continued to be strongly influenced by picrotoxin despite successive TAU applications. In these cells, a TAU-like inhibitory action could be restored by a baclofen application.

Such TAU effects are illustrated in Figure 1. As shown on the top trace, TAU totally abolishes the spontaneous activity of the cell after a 20 s delay. After 15 min, the cell is always completely silent. Rinsing is then performed by perfusing amphibian Ringer for ~3 min (second trace). Fifty-five seconds after the commencement of rinsing, spontaneous activity reappears and returns to its control level. Picrotoxin application (third trace) leads the cell to progressively increase its spontaneous firing rate until reaching a typical bursting mode (Duchamp-Viret *et al.*, 1993). During picrotoxin action, three successive applications of TAU are performed (fourth and fifth traces). They fail to counteract the picrotoxin-induced bursting mode. Fifty-eight minutes after picrotoxin application (bottom trace), baclofen was applied. It suppressed spontaneous firing until baclofen was rinsed, then allowing the picrotoxin-induced bursting mode to surge again (not shown).

In all cells where saclofen antagonized TAU inhibitory action (n = 8/11), successive applications of TAU (2–4 doses, totaling up to 50 µl) successfully restored TAU action. The action of saclofen and its antagonization by successive TAU applications are illustrated in Figure 2. As shown, TAU alone completely suppresses spontaneous firing and rinsing restores it. Spontaneous firing rate increases after saclofen applications until it reaches a plateau, as previously reported

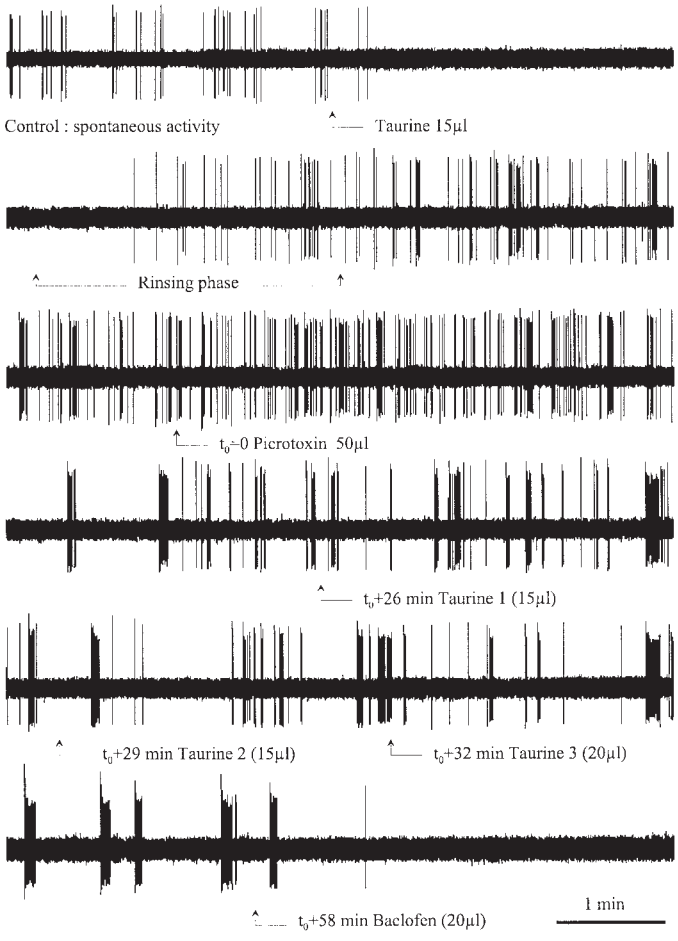

Figure 1 TAU and baclofen action under picrotoxin. The five traces are not in time continuity. Full explanations are in the text.

(Palouzier-Paulignan *et al.*, 2002). Then, the successive TAU applications succeed in counteracting saclofen action by decreasing and then completely suppressing spontaneous firing activity.

TAU action on mitral cell odor-induced activity

When the responses of the cells to the seven odorants systematically presented with the syringe in the TAU protocol were compared, no change of qualitative response profile was found in 98% of the cases (151 stimulation sequences). The only difference was an increased salience of the responses since they were evoked against a background of no spontaneous firing.

These results are illustrated throughout two series of responses to increasing odor concentrations that were obtained from the same cell before and after TAU application (Figure 3). Noteworthy was the drastic reduction of spontaneous firing that made the temporal response patterns particularly salient. In control conditions, cell response patterns showed classic progressive changes from threshold to maximal odor intensity (Duchamp-Viret *et al.*, 1989): namely, there was decrease in latency, shortening of burst duration and increase in discharge frequency. Under TAU, no evident alteration of odor responses occurred.

The increased salience of response patterns under TAU was specially noticeable for 17 stimulation sequences (10%) where TAU revealed a clear response pattern even though the odor response was almost indiscernible from the spontaneous activity in control conditions (Figure 4).

From a quantitative point of view, the curves obtained by plotting spike discharge frequency against odor concentration showed no change in the dynamics of odor intensity coding. The strength of the responses was not affected by TAU, as measured according to limits used in our previous studies (Duchamp-Viret and Duchamp, 1993; Duchamp-Viret *et al.*, 1993, 1997), where the criterion for a change in firing pattern after drug application was a change of at least 20% in spike frequency for the same odor concentration. This is shown in Figure 5. The two curves obtained in control condition and after TAU application are very similar. However, in the sample cell, as in 50% of recorded cells, TAU induced a slight sensitivity decrease, as shown by the higher response threshold (Figure 5).

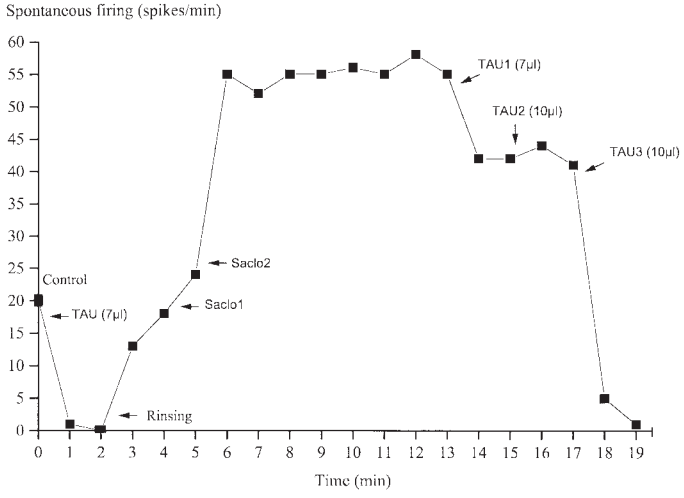

Figure 2 TAU action under saclofen. From left to right, the curve shows measures of spontaneous firing performed every minute during the continuous recording of the same cell in successive experimental conditions. Drug applications (indicated by arrows) were realized after the measures. The control spontaneous firing level was ~20 spikes/min. The first TAU application inhibited the cell within the first 2 min. The rinsing action restored rapidly the control level. Then, the two successive application of saclofen induced a slight increase of spontaneous firing rate. These saclofen applications counteracted the action of TAU. However, three successive applications of TAU antagonized the saclofen action and finally induced the disappearance of spontaneous firing again.

Discussion

Our results clearly show that TAU acts on mitral cells by lowering their spontaneous activity while fully maintaining their odor responsiveness or coding abilities, namely quality discrimination and intensity specification. However, some mitral cells (50%) showed a loss of their excitability at threshold and slightly suprathreshold odor concentrations, and a shift of their threshold one or two concentration steps forward. These observations are highly similar to those previously obtained with the $GABA_B$ agonist baclofen (Duchamp-Viret *et al.*, 2000). TAU action mechanisms are discussed according to two following hypotheses: TAU acts

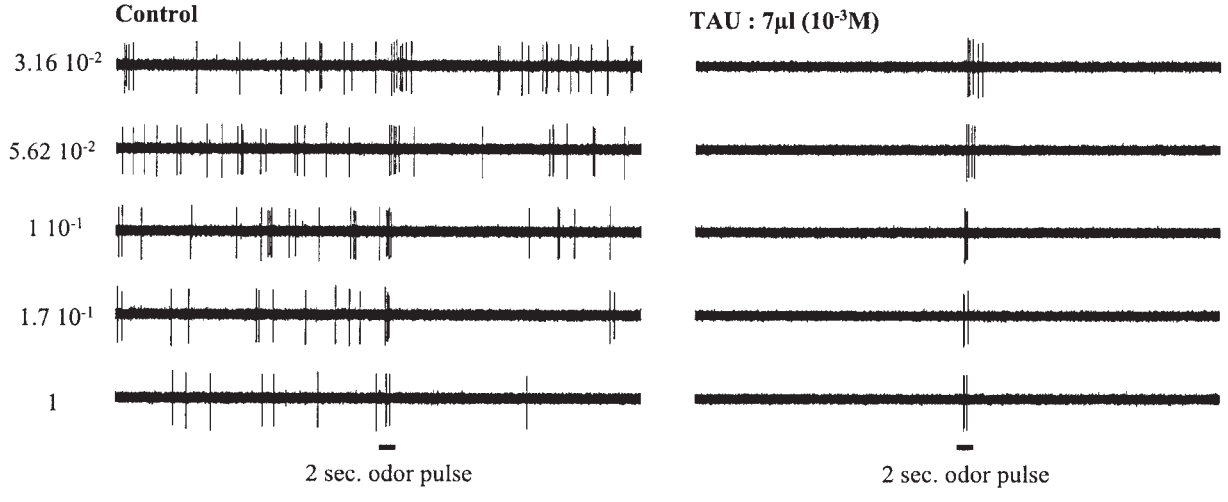

Figure 3 Series of temporal response patterns of the same mitral cell in control condition **(A)** and after TAU application **(B)**. The concentrations of the odor (limonene) are given in fraction of saturated vapor on the left the recordings, concentrations increasing from top to bottom. Dotted line: beginning of stimulus delivery, stimulus duration being 2 s.

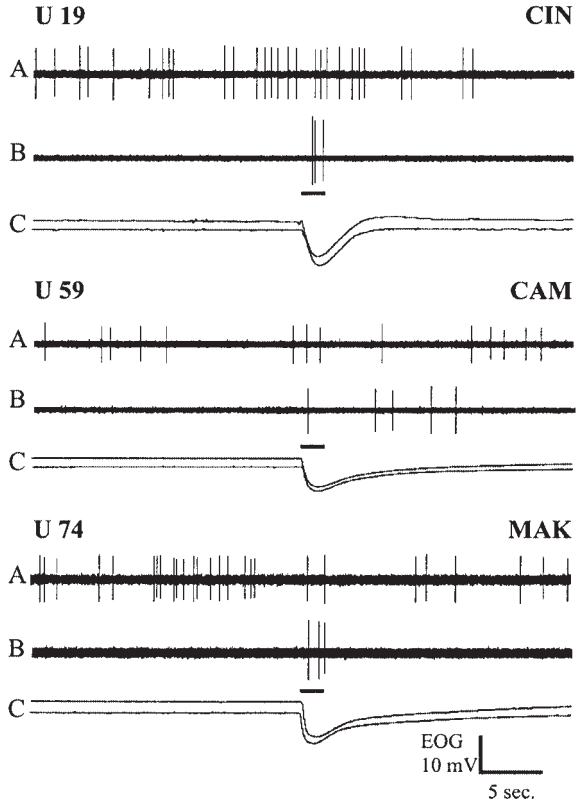

Figure 4 Examples of three mitral response patterns that were non-discernible in control conditions **(A)** and became salient under TAU action **(B)**. Even slightly modified, odor response patterns were maintained under TAU. Amplitudes of concomitant EOGs **(C)** revealed that the same odor at the same concentration elicited reproducible responses in control conditions and under TAU at the level of the cell population.

directly on GABA receptors or it modulates GABA release, and thus GABAergic inhibition strength exerted on mitral cells.

TAU action under picrotoxin

Picrotoxin blocks chloride channels, and thus chloride conductances through which GABA acts via $GABA_{A,C}$ receptors. Picrotoxin is not a specific receptor blocker, as is bicuculline, and its action is not reversible *in vivo*. However, it was preferred to bicuculline because bicuculline has too transient an action *in vivo*, and induces a very inconstant spontaneous firing pattern composed of silent periods alternating with bursting periods (Duchamp-Viret and Duchamp, 1993; Duchamp-Viret *et al.*, 1993). Using bicuculline, it would not have been possible to decide whether silent periods must be assigned to TAU or to bicuculline effects.

Picrotoxin effects on spontaneous activity observed in this study are in agreement with our previous results. Cells become highly active and develop a typical bursting firing mode (Duchamp-Viret *et al.*, 1993). Despite this high level of activity, TAU still abolishes or decreases cell spontaneous firing in 69% of the cells, compared with 90% under TAU

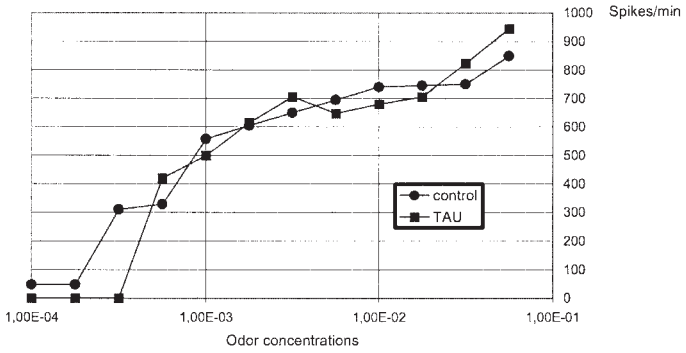

Figure 5 Concentration/response curves of the same mitral cell in control condition and after TAU application (7 µl at 5×10^{-3} M). In control condition, spontaneous firing rate was 48 spikes/min. Odor stimulus was isoamyl acetate. Comments are in the text.

alone. The strong disinhibition produced by picrotoxin may be sufficient to explain why additional doses of TAU did not affect four cells. However, in these cells, a direct baclofen agonization of $GABA_B$ inhibition was sufficiently potent to stop spontaneous firing, demonstrating that such an inhibition could occur despite a total block of chloride conductances. Thus, our study does not support the fact that TAU may act by setting in motion chloride conductances via $GABA_A$ or glycine receptors, as suggested in rats (Okamoto *et al.*, 1983; Hausser *et al.*, 1992; Quinn and Harris, 1995; Puopolo *et al.*, 1998). This discrepancy may be due to interspecific differences between rats and frogs.

TAU action under saclofen

Saclofen is a competitive reversible antagonist of $GABA_B$ receptors whose efficacy has been proved in the OB (Duchamp-Viret *et al.*, 2000; Palouzier-Paulignan *et al.*, 2002). Saclofen slightly increases the rate of spontaneous activity as previously reported in the frog *in vivo* (Duchamp-Viret *et al.*, 2000) and in the rat *in vitro* (Palouzier-Paulignan *et al.*, 2002). Such an action supports the tonic activation of $GABA_B$ receptors in the OB (Aroniadou-Anderjaska *et al.*, 2000). In addition, TAU efficacy drops to only 27% under saclofen. Thus, saclofen appears to preferentially prevent TAU action. This prevention is competitively antagonized by additional doses of TAU. This result may be interpreted according to two hypotheses: the successive doses of TAU displace saclofen and act directly on $GABA_B$ receptors, or the successive doses of TAU lead to an increase of GABA which competitively antagonizes saclofen.

Our results are consistent with the hypothesis that TAU acts by mimicking or by favoring GABA action on $GABA_B$ receptors. By contrast, measures of [^{3}H]GABA from synaptosomes or slices indicate that TAU would decrease GABA release rather than increase it (Namima *et al.*, 1982, 1983; Kamisaki *et al.*, 1993, 1996). However, it should be emphasized that these results are *in vitro* measures obtained indiscriminately from both neuronal and glial elements, which

cannot be easily placed within the physiological context of the bulbar network operating with odors *in vivo*.

Although we cannot state with certainty that TAU does not act at all via $GABA_A$ receptors, we can suggest that, even if this were the case, this way of action would not be the prevalent one. Interestingly, recent investigations performed in rats by Puopolo and coworkers (personal communication) have also shown a link between TAU and $GABA_B$. Indeed, they found that TAU would increase presynaptic inhibition via $GABA_B$ receptors and reduce mitral cell spontaneous activity. Our present results support such an hypothesis and further corroborate our previous finding describing $GABA_B$ agonist (baclofen) actions on mitral cell spontaneous activity (Duchamp-Viret *et al.*, 2000). Furthermore, in their personal communication, Puopolo *et al.* (1998) suggested that the same mechanisms would be involved in reducing mitral cell responses to olfactory nerve electrical shocks whereas we show that TAU acts mainly without altering mitral cell responses to odors. We have observed the same discrepancy between the rat *in vitro* and the frog *in vivo*. Baclofen decreased mitral cell responsivity to olfactory nerve electrical stimulation in the rat *in vitro* (Palouzier-Paulignan *et al.*, 2002), whereas it did not decrease mitral cell responsivity to odors in the frog *in vivo* (Duchamp-Viret *et al.*, 2000). It may be concluded that responses induced by odors and electrical shocks would lead the OB network to very different excited states, because electrical stimuli massively and nonspecifically excite the bulbar network.

Conclusion

In the OB of the *in vivo* frog, at rest or responding to odors, TAU appears to act via GABA receptors, and mainly via $GABA_B$ receptors. A direct action of TAU on $GABA_B$ receptor may be envisaged since the existence of baclofen-sensitive taurine receptors has been proposed. Indeed baclofen was shown as a fairly strong displacer of TAU binding (Kontro and Oja, 1985, 1990; Kontro *et al.*, 1990).

Our results do not allow us to distinguish between direct or indirect effects of TAU on $GABA_B$ receptors. However, several arguments suggest the indirect hypothesis. First, in electrophysiological studies the sole apparently direct effects of TAU that were reported were on chloride conductances, either via $GABA_A$ (Puopolo *et al.*, 1998) or glycine receptors (Hausser *et al.*, 1992; Hussy *et al.*, 1997). Secondly, TAU is largely accepted as a neuromodulator that can regulate either calcium fluxes in nerve terminals (Kuriyama *et al.*, 1978; Pasantes-Morales and Gamboa, 1980; Namima *et al.*, 1982, 1983) or the release of several neurotransmitters such as noradrenaline and acetylcholine (Kuriyama *et al.*, 1978), GABA (Kamisaki *et al.*, 1993). Thirdly, we have previously observed in the same experimental conditions that baclofen induces exactly the same effect as TAU (Duchamp-Viret *et al.*, 2000). Thus, TAU might act by enhancing GABA inhibitory mechanisms via $GABA_B$ receptors thanks to an increasing action of GABA release or to a decreasing action of re-uptake mechanisms. In the frog OB *in vivo*, Potapov (1985) reports that$GABA_B$ inhibitory action would be modulated by GABA glial uptake and release mechanisms. Given that TAU may be co-released with the primary neurotransmitter, namely glutamate, TAU might act in turn on glial and/or neural bulbar elements by stimulating GABA release or by decreasing GABA uptake within the glomerulus. The assumption that TAU action would be mediated via glial elements is reinforced by bibliographic data which establish that TAU, as well as GABA (Borden *et al.*, 1995; Gadea and Lopez-Colome, 2001a,b,c), is under control of uptake systems located in both neuronal and glial elements (Decavel and Hatton, 1995; Gragera *et al.*, 1995; Takuma *et al.*, 1996; Deleuze *et al.*, 1998; Chang *et al.*, 2001; Walz, 2002). In the OB, TAU may modulate GABA action mainly via $GABA_B$ receptors, the tight packaging of glomerulus elements facilitating such synaptic as well as extrasynaptic events. Regarding the integration of the olfactory signal, TAU action clearly reduces background activity in favor of the emergence of the odor-induced activity as does GABA action via $GABA_B$ receptors (Duchamp-Viret *et al.*, 2000; Isaacson and Vitten, 2003). Thus, the joint actions of TAU and GABA may favor the processing of the primary sensory information by increasing the signal to noise ratio in the frog olfactory bulb.

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