Research Report

Differential effects of iontophoretic application of the GABA_A-antagonists bicuculline and gabazine on tone-evoked local field potentials in primary auditory cortex: Interaction with ketamine anesthesia

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ABSTRACT

γ-Aminobutyric acid (GABA) is one of the main inhibitory transmitters in the central nervous system. In a recent study we have demonstrated differential effects of two iontophoretically applied GABA_A-blockers, bicuculline (BIC) and gabazine (SR 95531), on neuronal responses in primary auditory cortex (AI): Whereas the only effect of gabazine was to block GABA_A-mediated inhibition, BIC application additionally induced dose-dependent side effects, probably on calcium-dependent potassium channels. Here we investigated the effects of the two drugs on pure tone-evoked local field potentials (LFPs) in AI. In contrast to spiking activity, which reflects neuronal output, LFPs are believed to mainly reflect dendritic activity and therefore neuronal input. LFPs were recorded from the left AI of anaesthetized and unanaesthetized Mongolian gerbils before, during and after microiontophoretic application of BIC and gabazine using multi-barrel glass electrodes. After the application of both drugs, a significant increase of the amplitude of the N1 component of the LFP was observed in both anaesthetized and unanaesthetized animals, but this increase was significantly more pronounced after BIC than after gabazine application, a result which corresponds to the effects on neuronal discharge rate reported earlier. In contrast, the effects of BIC and gabazine on LFP duration (prolongation) and LFP spectral tuning (sharpening) were affected by ketamine anesthesia, an effect that was not seen in the spiking data. We conclude from the data presented that the main functional role of GABA_A-mediated inhibition in auditory cortex is to (1) prevent over-excitation (seizures) of cortical networks and (2) to speed up cortical processing.

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1. Introduction

γ-Aminobutric acid (GABA), one of the main inhibitory neurotransmitters in the central nervous system, is the major inhibitory neurotransmitter in cerebral cortex (Curtis and Johnston, 1974; Krnjevic, 1984; Winer, 1992). There it acts mainly on GABA_A- and GABA_B-receptors (Bormann, 1988, 2000; Chebib and Johnston, 1999). GABA_A-receptors mediate fast inhibitory postsynaptic potentials (Metherate, 1998; Li et al., 1996) and therefore are particularly well suited to generate or sharpen receptive field properties of cortical neurons.

In a recent study (Kurt et al., 2006), we have investigated differential effects of two iontophoretically applied GABA_A-blockers, bicuculline (BIC) and the pyridazinyl-GABA derivate gabazine (SR 95531), on single and multi-unit responses in primary auditory cortex (AI). We could demonstrate that, depending on the dose applied, BIC can have deleterious effects on neuronal response selectivity which do not reflect the loss of GABAergic inhibition. These effects include increased discharge rates of both spontaneous and evoked neuronal spiking activity, widening of spectral receptive fields as well as a prolongation of tone-evoked responses. We suggested that this effect may be due to non-GABAergic side effects of BIC reported in the literature which may affect neuronal discharges in a dose-dependent manner. These side effects include inhibition of GABA uptake, reduction of resting membrane conduction resulting in membrane depolarization, prolongation of calcium-dependent action potentials, paroxysmal depolarization shifts and apamin-like potentiation of burst firing (Olsen et al., 1976; Heyer et al., 1981; Johnson and Seutin, 1997). At least some of these secondary effects are thought to result from actions of BIC on calcium-dependent potassium channels (Johansson et al., 2001). In contrast, application of gabazine, although it has a higher affinity for the GABA_A-receptor than BIC, never led to observable side effects but only to blocking of GABA_A-mediated inhibition (see also Chambon et al., 1985; Heaulme et al., 1986; Hamann et al., 1988).

Here we investigated whether the deleterious effects of BIC on pure tone-evoked responses of single and multi-units in AI can also be seen on the population level of cortical activity. We therefore compared the effects of iontophoretic application of BIC and gabazine on pure tone-evoked local field potentials (LFP) in gerbil AI.

As LFP mainly reflect dendritic potentials and therefore are considered to represent neuronal input, a comparison of this report with the data presented in Kurt et al. (2006) may also allow for a differentiation of the effects of blocking GABA_A-mediated inhibition on neuronal input and (spiking) output, respectively. More specifically, LFP reflect both inhibitory as well as excitatory dendritic inputs (inhibitory and excitatory postsynaptic potentials, IPSP and EPSP, respectively), whereas spiking responses only reflect above threshold excitation of the neuron recorded from. Therefore, changes in LFP characteristics may be seen after blocking inhibitory dendritic inputs that are not necessarily reflected in spiking output.

2. Results

2.1. Database

All results presented are based on measurements on a total of 26 male Mongolian gerbils. Pure tone-evoked LFP were recorded at a total of 91 recording positions. Effects of application of GABA_A-antagonists on the N1 component of pure tone-evoked LFP were investigated at 38 recording positions in unanaesthetized animals (BIC: 27, gabazine: 11) and at 53 positions in anaesthetized animals (BIC: 17, gabazine: 36).

2.2. Drug effects on N1 spectral tuning

To characterize the spectral tuning of pure tone-evoked LFP, we used the measure of the $Q_{10\,db\,N1}$ (cf. Experimental procedures).

Fig. 1 shows two examples of this type of analysis: It can be seen that in both cases the N1 spectral tuning became sharper under the influence of GABA_A-blockers, leading to higher $Q_{10\,db\,N1}$-values, respectively.

Fig. 2 shows the population data of all recording sites tested. Here the effect could be found for the population data...
of both BIC (red, left) and gabazine (blue, right) groups. A statistical analysis revealed a significant increase in $Q_{10 \, \text{dB}_N1}$, and therefore a sharpening of spectral tuning of the N1 component, in all experimental groups (paired T-test $Q_{10 \, \text{dB}_N1}$ control vs. drug: unanaesthetized: BIC: $P=7.7e-4$, degrees of freedom ($df$)=25, gabazine: $P=8.3e-3$, $df=10$, anaesthetized: BIC: $P=0.032$, $df=18$, gabazine: $P=1.5e-3$, $df=14$). This effect was significantly stronger in unanaesthetized than in anaesthetized animals (right bar chart in Fig. 2, ANOVA: $P=0.034$, $df$ between groups: 1, $df$ within groups: 69). No difference in the effects of BIC vs. gabazine could be detected (ANOVA: $P=0.25$, $df$ between groups: 1, $df$ within groups: 69).

Interestingly, this result is in contrast to what we reported for the effects of BIC and gabazine on spectral tuning of neuronal spiking responses (cf. Kurt et al., 2006): There, no effect of blocking GABA_A-mediated inhibition on spectral tuning could be detected (cf. Discussion).

2.3. Drug effects on N1 amplitude

The most prominent effect of disinhibition induced by GABA_A-antagonists is an increase in neuronal activity. In spiking responses (neuronal output) this is true for both spontaneous as well as evoked activity (Kurt et al., 2006; Sillito, 1975). For LFP, a higher absolute amplitude for example of the N1 component (cf. Fig. 3, red) does not necessarily reflect higher neuronal activity, but could also result from higher synchrony of neuronal activity. Both effects, a higher level and a stronger synchrony of neuronal activity, could cooperatively or alone lead to a higher absolute amplitude of the LFP.

![Fig. 2](image)

Fig. 2 – Effects of drug application on LFP tuning sharpness. Scatter plots of individual data points (unanaesthetized: top; anaesthetized: middle) and population statistics (bottom bar charts) are given. Both BIC (left, red) and gabazine (right, blue) led to a significant increase in LFP tuning sharpness (=smaller frequency receptive fields), both in anaesthetized and unanaesthetized preparations. The effect was stronger in unanaesthetized than in anaesthetized preparations.

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Fig. 3 – Response parameters determined from LFP responses. From LFPs three main parameters were determined: The N1 amplitude (red), the N1 peak duration (=width of the N1 peak at 70% of N1 peak amplitude; blue) and the duration of the N1–P2 complex (green).

Fig. 4 gives examples of effects of BIC (left) and gabazine (right) on pure tone-evoked LFP in AI of unanaesthetized (top) and anaesthetized (bottom) animals. As can be seen in comparison to the control measurements (black curves), both BIC (red) and gabazine (blue) application results in enlarged N1 components. Although this effect could be of variable magnitude (cf. Fig. 4 top left vs. bottom right; Fig. 5 scatter plots), it was significant over the population of all recording sites tested in all animal groups (paired T-test N1 amplitude control vs. drug: unanaesthetized: BIC: P = 3.3e−7, df = 26, gabazine: P = 0.04, df = 10, anaesthetized: BIC: P = 1.8e−5, df = 16, gabazine: P = 2.2e−4; df = 35; Fig. 5 bottom, left bar chart). In addition, a comparison of the amplitude changes between all BIC and gabazine experiments showed that the effect on N1 amplitude was significantly more pronounced during BIC than during gabazine application (ANOVA: P = 0.05; df between groups=1, df within groups=89; Fig. 5 bottom, right bar chart).

2.4. Drug effects on N1 duration

In Fig. 4, it can be seen that application of BIC or gabazine may, in addition to the described effects on N1 amplitude, have an effect on the duration of the LFP: In the two examples shown from unanaesthetized animals (top), one has the impression that the pure tone-evoked LFP is longer under either drug condition than during control conditions. In the examples shown from anaesthetized animals such an effect cannot be seen (bottom), and this does not seem to correlate with strong or moderate amplitude changes (cf. Fig. 4 top left vs. bottom right). Indeed, when looking at N1 peak duration as a measure of LFP duration (cf. Fig. 3, blue), this effect is significant across the population of data points only in measurements on unanaesthetized animals, but not in anaesthetized animals (paired T-test N1 peak duration control vs. drug: unanaesthetized: BIC: P = 0.03, df = 25, gabazine: P = 0.004, df = 16, anaesthetized: BIC: P = 0.19, df = 16, gabazine: P = 0.26; df = 34; Fig. 6 scatter plots and bottom, left bar chart). Also a comparison of the mean N1 duration changes in anaesthetized vs. unanaesthetized animals, irrespective of type of applied drug, showed a significant difference (ANOVA: P = 0.01, df between groups=1, df within groups=66; Fig. 6 bottom, right bar chart).

An analysis of the drug-induced change of the duration of the N1–P2 complex as a second measure of LFP duration (cf. Fig. 3, green) led to similar results, although here the results for the unanaesthetized gabazine group showed only a trend that was not significant (paired T-test N1–P2 complex duration control vs. drug: unanaesthetized: BIC: P = 5.8e−5, df = 26, gabazine: P = 0.11, df = 10, anaesthetized: BIC: P = 0.16, df = 16, gabazine: P = 0.34; df = 23; Fig. 7 scatter plots and bottom, left bar chart). Again, as for the N1 peak duration, the comparison between mean N1–P2 duration changes in anaesthetized vs. unanaesthetized animals, irrespective of type of applied drug, showed a highly significant difference (ANOVA: P = 3.6e−5, df between groups=1, df within groups=77; Fig. 7 bottom, right bar chart).

2.5. Drug effects on cortical processing complexity

It was already stated above that LFP amplitude is, besides overall neuronal activity, highly affected by the synchrony of neuronal (dendritic) activity: The higher the synchrony, the higher the amplitude. Consequently, the roughness of the LFP time course should correlate with the complexity of neuronal processing: the smoother the LFP the higher should be the processing complexity, or vice versa the higher the roughness of the LFP the higher the synchrony of neuronal activity. From Fig. 4, one may get the impression that the smoothness of the LFP is bigger under drug conditions, i.e., under blocking of...
GABA\textsubscript{A}-mediated inhibition (cf. Fig. 4, e.g. top panels). Clearly this subjective impression will be strongly affected by overall LFP amplitude. To get an objective measure of LFP roughness that is independent of absolute LFP amplitude, we did the following analysis:

The mean LFP for each recording location after stimulation with BF\textsubscript{N1} was subtracted from the single trial measurements resulting in a dataset where only the non-phase locked stimulus-dependent and the stimulus-independent amplitude fluctuations of the single trials were conserved. Then the mean amplitude of each single trial was subtracted from this trial, eliminating DC-offsets that might have been different between the trials, resulting in stimulus- and DC-offset-independent amplitude fluctuations of the single trials. Finally, the standard deviation over all trials were calculated and served as a measure of LFP roughness.

Fig. 8 shows the results of this analysis. As it turned out, blocking of GABA\textsubscript{A}-mediated inhibition by either BIC or gabazine led to a highly significantly increased roughness in most groups, except for the anaesthetized gabazine group, where the changes were not significant (paired T-test LFP roughness control vs. drug: unanaesthetized: BIC: \( P = 3.6 \times 10^{-9} \), gabazine: \( P = 0.91 \)).
For the significant groups, these results mean that blocking GABA_A-mediated inhibition leads to a higher synchrony in addition to an overall higher activity of dendritic neuronal activity as demonstrated in Fig. 5.

3. Discussion

3.1. Effects of bicuculline and gabazine on pure tone-evoked neuronal responses: comparison of effects on spiking vs. LFP responses

In a previous study (Kurt et al., 2006), we described the effects of BIC and gabazine on neuronal discharge responses in primary...
auditory cortex. We reported that blocking GABA_A-mediated inhibition by either BIC or gabazine led to (1) an increase in spontaneous and stimulus-evoked discharge rate, (2) a prolongation of response duration, but (3) no change in spectral receptive field width. Only after application of high doses of BIC a widening of spectral receptive field width could be observed, but we could demonstrate that this effect was not due to blocking of GABA_A-mediated inhibition but rather was due to side effects of BIC on calcium-dependent potassium channels (cf. Johansson et al., 2001). All these effects were observed similarly in both anaesthetized and unanaesthetized preparations, and therefore all data from both preparations were pooled in this study.

In the present paper, based on an analysis of pure tone-evoked LFP, we presented comparable measures of neuronal activity (N1 peak amplitude), response duration (N1 peak duration and N1–P2 duration) and spectral tuning ($Q_{10\,\text{dB}}$). Out of these, only the measure of neuronal activity gave results corresponding to the study on spiking responses: Application of both BIC and gabazine led to a significant increase in N1 peak amplitude (Fig. 5) which corresponds to the increase in evoked spiking activity as reported in Kurt et al. (2006). The fact that the increase in N1 peak amplitude that was induced by BIC application was significantly larger than the gabazine-induced effect was also seen as a trend in the spiking responses and is likely to be due to the described side effects of BIC.

In contrast to this correspondence of the results reported by Kurt et al. (2006) and the present study, the LFP measures of
response duration and spectral tuning gave results deviating from the data on spiking responses in some details.

First of all, the effect of a prolongation of response duration after blocking of GABA<sub>A</sub>-mediated inhibition as reported for the spiking responses was also seen in both LFP measures of response duration (Figs. 6 and 7), but this effect was only observed in unanaesthetized animals and not in anaesthetized animals. This observation points to an interaction between the anesthetic used in our study and the GABA<sub>A</sub>-receptors or antagonists used (cf. below).

Second, and in contrast to the spiking data, we observed an effect of blocking GABA<sub>A</sub>-mediated inhibition on spectral tuning, namely a sharpening of the spectral receptive field as given by the Q<sub>10 dB_N1</sub> (rather than the widening of spike–response-based spectral receptive fields observed during high doses of BIC) in all groups tested. Furthermore, in unanaesthetized preparations, blocking of GABA<sub>A</sub>-mediated inhibition had significantly stronger effects on spectral tuning that led to a more pronounced sharpening of LFP spectral tuning compared to that in anaesthetized preparations.

Fig. 8 – Effects of drug application LFP roughness. Scatter plots of individual data points (unanaesthetized: top; anaesthetized: middle) and population statistics (bottom bar charts) are given. Both BIC (left, red) and gabazine (right, blue) led to a significant (except for the anaesthetized gabazine group) increase in LFP roughness in both anaesthetized and unanaesthetized preparations. The effect was significantly stronger during BIC than during gabazine application and also significantly stronger in unanaesthetized compared to anaesthetized animals (bottom right panel).
This latter discrepancy between spiking responses and LFP responses may be due to the different nature of these two measures of neuronal activity: Spiking responses are the output function of neuronal activity and are always based on neuronal excitation. In contrast, LFP responses are based on dendritic inputs and can be both excitatory or inhibitory, and these two components cannot be distinguished by simply examining the LFP waveform, because this waveform strongly depends on a number of factors, e.g. cortical layer of recording. Hence, blocking GABA\textsubscript{A}-mediated inhibition may eliminate the inhibitory part of the LFP, leading to a decreased width of LFP spectral receptive field. That is, we here observe an effect on sub-threshold inhibitory input to neurons that cannot be seen in above-threshold excitatory spiking neuronal output.

Although this may explain the differences between data from spiking vs. LFP responses, it does not yet explain the observed differences between anaesthetized and unanaesthetized preparations.

### 3.2. Effects of ketamine anesthesia on GABA\textsubscript{A}-mediated neuronal transmission

Effects of BIC and gabazine on two of the LFP measures investigated here were affected by anaesthesia, namely the response duration as expressed by N\textsubscript{1} peak duration and N\textsubscript{1}–P\textsubscript{2} duration and the spectral LFP tuning width as given by Q\textsubscript{0} dB N\textsubscript{1}. Obviously there seems to be some interaction between the anesthetic used here and the GABA\textsubscript{A}-receptors or antagonists applied. The anesthetic used in our study was a mixture of ketamine, a non-competitive NMDA receptor antagonist, and xylazine, an \(\alpha\delta\)-adrenergic agonist.

GABA\textsubscript{A}-receptors are GABA-gated Cl\textsuperscript{-} channels composed of 5 out of 19–20 known subunits (cf. Chebib and Johnston, 1999; Bormann, 2000; Hevers et al., 2007). The composition of subunits may differ strongly between species, brain region and developmental state and different GABA\textsubscript{A}-receptor subtypes may therefore express different pharmacology (Endo and Olsen, 1993; Nguyen et al., 1995). Although it was long believed that there are no effects of ketamine on GABA\textsubscript{A}-receptors (despite known effects of other anesthetics on GABA\textsubscript{A}-receptors, e.g. Ueno et al., 1997), a recent study by Hevers et al. (2007) provided evidence that ketamine may modulate and directly activate at least two subtypes of GABA\textsubscript{A}-receptors, namely those composed of the subunit combinations \(\alpha6\delta2\gamma\) and \(\alpha6\delta3\sigma\) which are expressed in cerebellar granule cells (Quirk et al., 1994). Although these GABA\textsubscript{A}-receptor subtypes so far are not found to be expressed in cortex, the finding of Hevers et al. (2007) may point to a direct influence of ketamine on the GABA\textsubscript{A}-receptors in cortex also. There, \(\alpha4\delta\gamma\) and \(\alpha4\delta\sigma\) subtypes are known to be expressed (Benke et al., 1997), so at least the \(\delta\)-subunit which plays a role in the ketamine-influenced subtypes in cerebellum is also expressed in cortical subtypes. On the other hand, Hevers et al. (2007) report that at least the \(\alpha4\delta2\gamma\) subtype is not affected by ketamine, but still the data presented in our present report suggest that there has to be a GABA\textsubscript{A}-receptor subtype in AI which is affected by ketamine.

If so, one might be able to understand the differences we observed in anaesthetized vs. unanaesthetized animals during application of BIC or gabazine, respectively, if we suppose a similar effect of ketamine on the GABA\textsubscript{A}-receptor in AI as the one reported by Hevers et al. (2007) for cerebellar granule cells; that is, a potentiation of the GABA effect on the GABA\textsubscript{A}-receptor. Possibly under the influence of ketamine, the effect of GABA on the GABA\textsubscript{A}-receptor cannot be blocked as efficiently as in the unanaesthetized preparation. For our data on LFP response duration, this would explain why we saw no prolongation of response duration during ketamine anesthesia, because there GABA would still be effective to a certain extent which prevents a disinhibition strong enough to significantly prolong the response (as seen in the unanaesthetized preparation). The same line of arguments may explain our data on spectral LFP tuning, where we saw a smaller sharpening effect on spectral receptive fields during ketamine anesthesia under the influence of the GABA\textsubscript{A}-antagonists BIC and gabazine. Again, if blocking of GABA\textsubscript{A}-mediated inhibition was less effective under the influence of ketamine, this would explain the smaller effect on LFP tuning during anesthesia.

Although these interpretations are still highly speculative, we believe that our data do suggest an interaction between ketamine anesthesia and GABA\textsubscript{A}-antagonistic drug effects.

The alternative interpretation, that the observed effects of anesthesia are due to xylazine, is unlikely, since xylazine mainly has cardiovascular (depression of heart rate) and respiratory effects (respiratory depression).

### 3.3. Effects of blocking GABA\textsubscript{A}-mediated inhibition on cortical processing complexity

In Fig. 8, we demonstrated that blocking GABA\textsubscript{A}-mediated inhibition leads to an increased roughness of the LFP time course. In a paper by van Drongelen et al. (2003), it is reported that the increased population activity during BIC-induced seizure-like activity in mouse cortical slices is, at least in this preparation, due to an increased recruitment of neurons rather than to increased synchrony of the individual neurons. So, in principle, based on our analysis of LFP roughness, we cannot unambiguously decide whether the increase in LFP roughness we observed is due to an increased neuronal synchrony, increased neuronal recruitment or both. Nevertheless, we find it likely that higher synchrony of the stimulus-driven dendritic activity may at least contribute to the effect observed here; that is, the complexity of cortical neuronal processing is decreased by blocking GABA\textsubscript{A}-mediated inhibition. Although we found quantitative differences between BIC and gabazine application as well as between anaesthetized and unanaesthetized preparations, the effect seems to be qualitatively present under all conditions tested. As inhibition in general allows for shorter processing cycles because excitatory events can be terminated faster so that the neuron can discharge at higher rates (cf. Grothe and Klump, 2000), i.e., can perform more processing steps per time, an increased synchrony of stimulus-driven neuronal activity after blocking inhibition is what one would expect. In other words, intact inhibitory interactions are crucial for high processing rates of single neurons and therefore high processing complexity of cortical networks.
4. Conclusion

Based on the results reported here and those presented in Kurt et al. (2006), we believe that the role of GABA_A-mediated inhibition in auditory cortical networks is to introduce subtle inhibitory inputs to cortical neurons to prevent (1) over-excitation (which could lead to seizures) by reducing both amplitude and duration of neuronal responses. The latter effect consequently leads to shorter single responses and therefore allows for (2) higher processing rates of single neurons. At the network level, this seems to be crucial for fast neuronal communication; that is, GABA_A-mediated inhibition speeds up information processing in cortical networks. In contrast, the effect on spectral receptive field shape of this type of GABAergic inhibition, although possibly still present at the cortical input level, seems to be of minor functional relevance for the output functions of auditory cortical neurons.

5. Experimental procedures

Experiments were performed on anesthetized (n=12) or unanesthetized (n=14) adult male Mongolian gerbils (Meriones unguiculatus) weighing 80–120 g. Animal preparation, microiontophoresis, and acoustic stimulation were carried out as described in the preceding paper (Kurt et al., 2006) and will therefore only be given here in short.

5.1. Animal preparation

Surgery was performed under deep general (halothane, Sigma) and local anesthesia (Gingiain, Aventis). Body temperature was maintained at 37 °C using a remote-controlled heating blanket. Prior to surgery, ear canals and the tympanic membranes were inspected and found to be free of disease. The left auditory cortex was exposed by craniotomy, leaving the dura intact.

For acute experiments on anesthetized animals, the animal's head was stereotaxically fixated during recordings by a 2.5-cm-long rectangular aluminum bar fixed to the frontal bones (head anchor). Insect needles that were inserted into the skull served as reference electrodes. Measurements were carried out in an anechoic, sound-attenuated chamber. Throughout the experiments, anesthesia was maintained by an intraperitoneal infusion of ketamine (50 mg/ml; Ratiopharm), xylazine (Rompun 2%, BayerVital) and isotonic sodium chloride solution (mixture 9:1:10) as reference electrodes. Measurements were carried out in an anechoic, sound-attenuated chamber. Throughout the experiments, anesthesia was maintained by an intraperitoneal infusion of ketamine (50 mg/ml; Ratiopharm), xylazine (Rompun 2%, BayerVital) and isotonic sodium chloride solution (mixture 9:1:10) at a rate of 0.06 ml/h. Body temperature was again maintained at 37 °C. At the end of a recording session (which typically lasted 20 to 24 h), animals were killed by an intrapulmonary injection of T61 (Intervet).

For experiments on unanesthetized animals, a chronic preparation with a plastic cylindrical chamber (diameter 6.5 mm) with a screw cap that was attached to the skull over the trepanation area was used in addition to the head anchor. Following surgery, the dura was covered with an antibiotic paste (Volon, Dermapharm, and Grünwald) and the plastic cylinder was sealed with the screw cap. Animals were allowed a recovery period of 2 days before the first recording session began. On recovery, animals showed completely normal behavior, with no signs of distress. Animals were habituated to sit quietly during recordings that were made in the same recording chamber as that used for anaesthetized animals. This type of chronic preparation was stable over a period of 4 to 6 weeks. To minimize animal stress, each recording session in unanaesthetized animals was limited to 3–5 h. Between recording sessions, animals showed no sign of stress or impairment due to the head anchor.

5.2. Microiontophoresis

Microiontophoretic administration of drugs was performed with an Iontophore-3 iontophoresis unit (Science Products) via three-barrel or five-barrel micropipettes (tip diameter of 9–18 μm and 15–30 μm, respectively). Micropipette barrels were filled with a combination of the following solutions: 3 M NaCl for extracellular recording and for automatic current compensation, BIC (bicuculline methiodide; 10 mM, pH 3.0), and gabazine (SR 95531; 3 mM, pH 3.0). All drugs were obtained from Sigma. Ejecting currents ranged from 15 nA to 80 nA, and the retaining current was always –15 nA. Under microscopical control, the micropipette assembly was stereotaxically inserted through a small dural puncture and lowered into AI using a motorized stepping microdrive. Sites for pipette penetrations (spaced about 100–200 μm apart) were chosen in order to evenly sample the cortical surface whilst avoiding injury to blood vessels. Penetrations were made tangential to the cortical surface such that the pipettes remained for long distances in the middle layers of AI.

5.3. Recording of local field potentials (LFPs)

Neuronal population activity was recorded using a multichannel recording system (Multichannel Acquisition Processor, Plexon), where the signal from the micropipette was amplified (1000–5000×), filtered (3–170 Hz band-pass) and continuously stored for offline analysis with a sampling rate of 2000 Hz. In addition, time stamps of acoustic stimulation relative to LFP recording time were stored.

5.4. Acoustic stimulation

Acoustic pure tone stimuli were delivered free field via a speaker mounted approximately 2 cm in front of the animal's head. Speaker output was measured prior to an experiment using a 0.5-in. condenser microphone (Bruel and Kjaer model 4133) placed at the position of the animal’s head and facing the speaker. The signal from the amplifier was continuously monitored during the experiments. For frequencies between 0.3 and 20 kHz, the output of the speaker (STAX SRM-1/MK-2) was found to be flat within ±5 dB and without measurable (with Bruel and Kjaer 2033) distortion up to 90 dB sound pressure level (SPL); that is, any potential distortion were ≥90 dB below signal level. All stimuli were presented at a constant intensity of 65±5 dB SPL and had a duration of 200 ms with 5-ms rise and fall times. Inter-stimulus intervals used were 0.5 s.
Neuronal population activity was recorded in the primary auditory cortex field AI. Verification of recording site was based on the known functional organization of the auditory cortex of the Mongolian gerbil (tonotopic gradients, temporal response characteristics of single- or multi-unit activity-like latency and temporal response pattern that was recorded parallel to the LFP recording) as documented in the literature (cf. Thomas et al., 1993; Schulze et al., 1997). At each recording site, pure tone frequencies ranging from 0 to 20,000 Hz were presented in variable steps. To test the pharmacological effects of BIC and gabazine on pure tone-evoked LFPs, responses were recorded before (pre-drug), during (drug) and after (recovery) continuous iontophoresis of either BIC or gabazine. Stimuli were presented in random order. Each complete set of stimuli was presented 15 times, and stimuli were randomized separately for each presentation. Drugs were typically applied for between 6 and 14 min, which was the time required for the presentation of one complete set of stimuli. Following termination of drug application, stimuli were presented repeatedly (for up to ~45 min) until response magnitude and selectivity returned to pre-drug levels.

### 5.6. Data analysis

A number of parameters were determined from measurements of LFPs. These parameters were defined concerning to response parameters of single and multi-unit responses to allow for a comparison of the results of these two methods (cf. Kurt et al., 2006).

For each stimulus and recording condition, mean LFPs were calculated from the measured single trial LFP recordings (Fig. 3, black line). From these, the amplitude of the N1 component (as a measure of response strength; Fig. 3, red), the duration of the N1 peak (=width of the N1 peak at 70% of N1 peak amplitude; Fig. 3, blue) and the duration of the N1–P2 complex (Fig. 3, green) were determined. These latter two served as measures of response duration.

A plot of the N1 amplitude as a function of pure tone frequency (=N1 frequency response function=FRF<sub>N1</sub>; Fig. 1 insets) was used to evaluate the spectral tuning of the LFP response. From these plots, the N1 best frequency (BF<sub>N1</sub>=pure tone frequency that evoked the largest N1 amplitude) and a response. From these plots, the N1 best frequency (BF<sub>1</sub>=pure insets) was used to evaluate the spectral tuning of the LFP calculated from the measured single trial LFP recordings (Fig. 3, N1 peak (=width of the N1 peak at 70% of N1 peak amplitude; Fig. 3, red), the duration of the response magnitude and selectivity returned to pre-drug levels.

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