Achse gedreht. Bei gleichem Schalldruck wurde anschliessend der Freifeldpegel L_F mit derselben Sonde gemessen. Der Unterschied zwischen Freifeld- und maximalem Gehörgangspegel ist dann $\Delta L_{GF} = L_{Gmax} - L_F$. Aus den in[2] angegebenen Hörschwellenpegeln L_{HSF} im Freifeld ergeben sich damit die Hörschwellenpegel im Gehörgang zu $L_{HSG} = L_{HSF} + \Delta L_{GF}$.

Die Ergebnisse gibt die Figur wieder. Der maximale Gehörgangspegel L_{Gmax} wird bei einem Einfallswinkel von ca. 45° gegenüber der Frontalrichtung gefunden.

Aus den Hörschwellenpegeln im Gehörgang L_{HSG} und der Übersprechdämmung ΔL aus[1] (Mittelwert von 3 Katzen) ergeben sich die zulässigen Maximalpegel im Gehörgang $L_{Gzul} = L_{HSG} + \Delta L$, die ebenfalls in der Figur angegeben sind. Sie dürfen nicht überschritten werden, wenn eine überschwellige Erregung des contralateralen Ohres ausgeschlossen werden soll. Bei Versuchen im stereotaktischen Gerät muss darauf geachtet werden, dass die Übersprechdämmung nicht durch Körperschallleitung in der Apparatur erheblich absinkt[4].

Summary. A report is given of the sound pressure levels which must not be exceeded in order to avoid suprathreshold interaural crosstalk during the stimulation of the cat auditory system by means of earphones.

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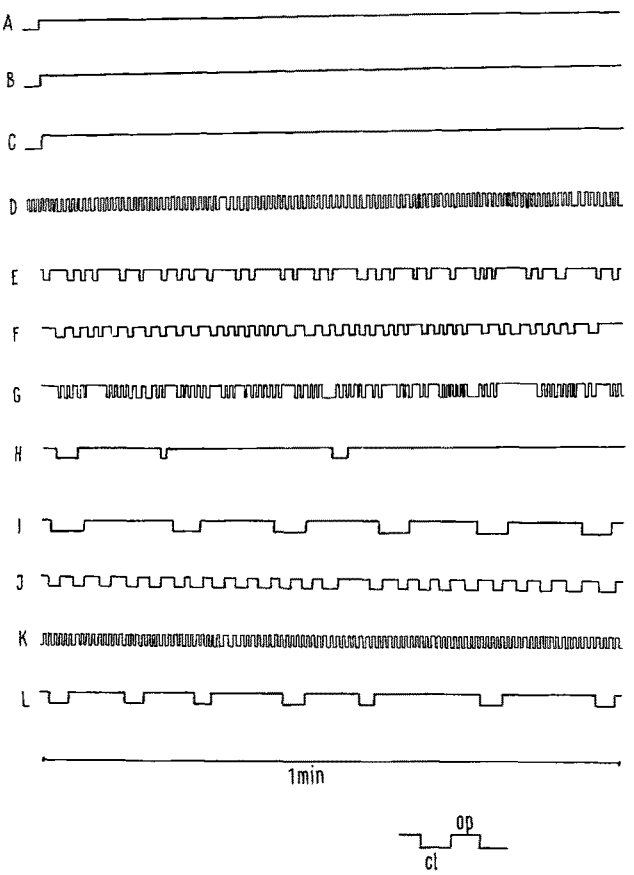
[4] Mit Unterstützung der Deutschen Forschungsgemeinschaft.

Effects of Fumigants on the Spiracles and the Possible Role of a Neurohumor on the Spiracular Activity of *Periplaneta americana* (L.)

Several attempts have been made to locate the focal centres of respiratory activity in insects. Although a number of workers have tried to identify the nervous centres which control respiration, the role played by different parts of the nervous system is far from clear. It has been suggested that thoracic[1,2] and abdominal[3,4] ganglia are important as pacemakers. However, the precise role of cerebral and suboesophageal ganglia has not been investigated. MYERS and FISK[3] have shown that decapitation removes all inhibition from the respiratory centres to cause constant breathing movements in *Byrsotria fumigata* (Guerin), which otherwise shows periodic ventilation movements. Decapitation causes increased carbon dioxide production in grasshopper nymphs[5].

During the course of studies on the effects of several fumigants on the physiology of insect respiration, it was noticed that the fumigants which are approximately equally lethal but physiologically different may be classified as inhibitory or excitatory as based on their action on respiration. Of the several fumigants tried, nicotine and a mixture of ethylene dichloride and carbon tetrachloride (EDCT) have been dealt with here as an example of antagonistic inhibitory and excitatory action, respectively, on the brain.

Spiracular activity was studied by the methods used by BHATIA and TONAPI[6] on intact, decerebrated and decapitated cockroaches of the species *Periplaneta americana* (L.). Ventilation movements of the abdomen and valvular movements of first and second thoracic spiracles were recorded. The activity of the second thoracic spiracle, whose valvular movements are synchronized with ventila-



Kymographic records of the spiracular activity in response to nicotine (A–D), EDCT (E–H) and untreated (I–L). (A) nicotine on intact insect; (B) nicotine on decerebrated insect; (C) nicotine on decapitated insect (D); nicotine released on the brain; (E) EDCT on intact insect; (F) EDCT on decerebrated insect; (G) EDCT on decapitated insect; (H) EDCT released on the brain; (I) intact insect in Ringer's solution only; (J) decerebrated insect; (K) decapitated insect; (L) effect of brain extract on decapitated insect (compare with I). cl, closing; op, opening of the spiracle.

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tion movements even when treated with fumigants, is described.

Nicotine when introduced in Ringer's solution flooding the insect's body[6] showed complete inhibition of spiracular movements of intact, decerebrated and decapitated insects (Figure A, B and C). The second thoracic spiracle remained open with nicotine solution, indicating that nicotine brings about inhibition in the pacemakers situated in the body outside the cephalic region. But when nicotine solution was released on the brain, increased respiratory movements of the spiracles (103 beats/min) were observed (Figure D), just as in the case of the decapitated insect. Obviously, nicotine diminishes the inhibitory action of brain.

EDCT in a similar test showed increased spiracular movements with intact (30 beats/min), decerebrated (40 beats/min), and decapitated (80 beats/min) insects (Figure E, F and G), thus exhibiting its excitatory effect on pacemakers. But when released on the brain it caused reduced spiracular activity (3 beats/min) (Figure H). The reactions of EDCT are diametrically opposite to that produced by nicotine.

Decerebration increased spiracular activity (23 beats per minute) and decapitation resulted in further increase (104 beats/min) (Figure J and K). This indicates that brain inhibits spiracular movements. Both cerebral and suboesophageal ganglia are inhibitory in action. This was further confirmed by releasing brain extract (5 brains homogenized in 0.5 ml and diluted in 200 ml of Ringer's solution) on the decapitated insect. The increased spiracular activity resulting from decapitation was inhibited to almost normal (Figure I and L), indicating that an unidentified neurohumor is involved in the spiracular regulation of respiration[7].

Zusammenfassung. Es wurde die Wirkung von Nikotin und EDCT auf die spirakulare Aktivität von *Periplaneta americana* (L.) untersucht. Nikotin hat eine hemmende und EDCT eine aufregende Wirkung auf das Gehirn. Untersuchungen an intakten, dezerebrierten und dekapitierten Insekten haben gezeigt, dass das Gehirn als Zentrum der Atmungsinhibition wirkt. Versuche mit Gehirnextrakten bei dekapitierten Insekten beweisen die hemmende Rolle des Gehirns und zeigen, dass ein unbekanntes Neurohormon für die spirakulare Aktivität des Insektes von Bedeutung sein könnte.

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An Osmiophilic Substance in Brain Synaptic Vesicles not Associated with Catecholamine Content

Autoradiographic studies of catecholamine-storing nerve endings of the rat brain[1,2] have indicated that despite retention of more than 50% of the radioactivity after glutaraldehyde-OsO_4 double fixation, no osmiophilic material is seen within the small synaptic vesicles (400 to 600 Å) by electron microscopy. Although larger vesicles (800–1000 Å) within the autoradiographically labeled nerve endings do exhibit osmiophilic granular material, the relative electron-opacity of the material does not fluctuate in proportion to monoamine content of the brain after pharmacological elevations or depletions of monoamine stores[3,4].

For peripheral autonomic nerves, the amount of electron-opaque material within synaptic vesicles correlates well with the catecholamine content of the nerves as judged by biochemical, fluorescent histochemical and autoradiographic[5–7] assays. Glutaraldehyde pre-fixation appears to result in better demonstration of osmiophilic vesicular material than is seen with only OsO_4 fixation[8,9]. Immersion fixation with cold 3% $KMnO_4$ is reported to result in the highest frequency of staining of the intravesicular material within sympathetic nerve endings[7]. Brain fixed with $KMnO_4$ has also been reported to reveal an intravesicular material within brain synaptic vesicles of the rat median eminence and locus coeruleus[10]. However, this fixative is difficult to use, since penetration of tissue is slow and tissues are difficult to section.

Since glutaraldehyde fixation of brain is associated with monoamine retention by autoradiography, but does not reveal electron-opacity in the small synaptic vesicles, the morphological discrepancy between the results of glutaraldehyde-OsO_4 fixation and $KMnO_4$ fixation do not seem to be directly explicable simply on the basis of better retention of vesicle catecholamine content. The present experiments have investigated the inability of glutaraldehyde-OsO_4 fixation to produce the synaptic vesicle electron-opacities revealed in the brain with $KMnO_4$: the vigor of reaction conditions has been varied to uncover possible physico-chemical differences in the reactivity of the 2 oxidants with intravesicular substances.

Materials and methods. Normal rats, rats pre-treated with reserpine (2.5 mg/kg, s.c., 18–25 h) or pargyline (100 mg/kg, i.p., 20 h) and rats given 35 μC H^3-norepinephrine (sp. act. 6.5 C/m*M*) by injection into 1 lateral cerebral ventricle (2 h) were used. Brains were fixed by perfusion with 5% glutaraldehyde (phosphate-buffered, pH 7.4). Adjacent tissue blocks were exposed to solutions of 1% OsO_4 or 3% $KMnO_4$ for a variety of times and at

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