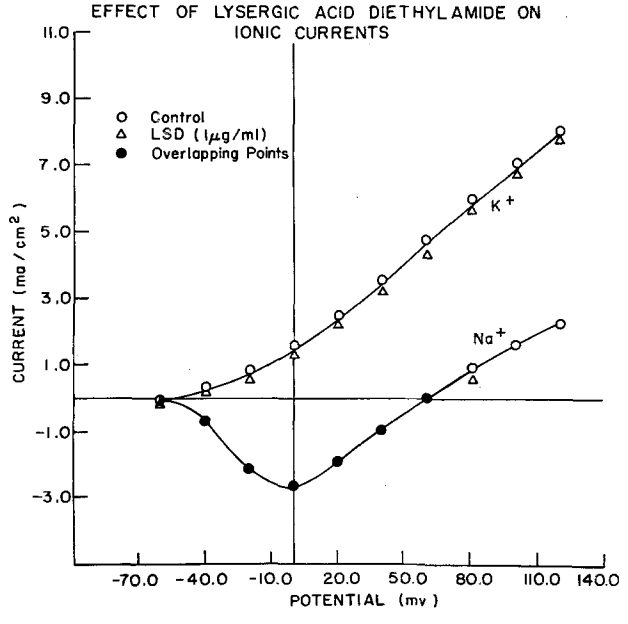
Ineffectiveness of Lysergic Acid Diethyl Amide-25 (LSD) on Altering Na-K Currents in Squid Giant Axon

Lysergic acid diethylamide-25 (LSD) is a potent hallucinogen which affects several aspects of neural activity and behavior in man and other animals. However, the mechanisms through which the effects on neural activity occur have not been well-defined. In an effort to evaluate the action of this hallucinogen on one aspect of neural function, its effects on the basic ionic events which give rise to the neuraxon potential in squid giant axon were investigated.

Methods. Squid giant axons were excised, freed of supporting tissue and other nerve fibres and placed into a chamber for measurement of ionic currents using the conventional voltage clamp technique[1]. Hepes buffer[2] in K-free artificial seawater at pH 7.4 at 4 °C was used to maintain the excised axon. The transient peak and steady state currents were measured using step pulses. Estimation of the leakage current by a linear extrapolation was obtained by using hyperpolarizing pulses. K and Na currents were obtained by subtracting the estimated leakage current for each step pulse from the steady state and transient peak currents, respectively. The conventions used to represent these data are a) all positive currents are outward currents and b) when the membrane potential is negative the internal potential is negative with respect to the external potential.

These currents were measured alone in K-free artificial seawater under control or baseline conditions and in the presence of LSD-25, 1 μg/ml, for periods of at least 10 min. LSD was obtained from the Center for Studies of Narcotic and Drug Abuse as D-lysergic acid diethylamide, Sandoz Pharmaceuticals, Hanover, New Jersey, batch No. 69003, as the tartrate, 0.1 mg/ml/ampule. This drug was documented to be 99% pure (analytical data sheet No. 15886–17).

Results. The effects of LSD-25 on the Na K currents is shown in the Figure. The Na-K currents are plotted on the ordinate as a function of the membrane potential which is plotted on the abscissa. There is no difference in the measured currents upon the addition to the external solution of either LSD-25 (Figure).



Effect of LSD-25 on Na-K current of squid giant axon. The currents are plotted on the ordinate, the membrane potential on the abscissa. The arrows indicate the experimental potentials at which the ionic currents were measured.

Discussion. These results demonstrate that administration of massive concentration of LSD do not alter the basic ionic events which give rise to the action potential. Nevertheless, single neuronal units in the midbrain raphe nuclei of rat exhibit significant inhibition of spontaneous firing rate after parenteral administration of minute amounts of LSD (10–20 μg/kg)[3]. Similarly, parenteral administration of small amounts of LSD in cat has been shown to produce inhibition of some classes of cerebral synapses[4,5] although not in all[6] and to alter significantly synaptic delay in the lateral geniculate system[7]. In man, administration of this drug in concentrations of 1–2 μg/kg has been shown to inhibit cortical dendritic activity[7] and synaptic transmission in the visual system[8]. Neural activity and behavior in lower species such as lobster[9], fish[10,11], salamander[12], snail[13], and spider[14] are also affected by this drug. Minute concentrations of LSD (0.0002 μg per ml) have also been observed to affect brain cells in tissue culture[15]. The behavior of the intact, free swimming squid in the presence of a concentration of LSD-25 1/100 as concentrated as that which was ineffective in altering the action potential of the giant axon (i.e., 0.001 μg/ml, free swimming; 1 μg/ml in giant axon preparation) significantly depressed spontaneous activity and swimming and depressed responsiveness to touch. This depression in spontaneous activity with LSD-25 is similar to that observed in the salamander[12] at concentrations of LSD-25 which were not too dissimilar. These results suggest that LSD-25 is effective in altering some aspects of neural activity in the squid but it is ineffective in altering the basic ionic events which give rise to the action potential. LSD does affect synaptic conduction, one effect being its blocking action of serotonergic post-synaptic receptor sites. Effects in several synapses have been previously observed[5,6,8].

Although LSD did not alter the Na-K currents in the squid giant axon harmaline, another hallucinogen, decreased the sodium membrane conductance of squid giant axon[16]. Although Δ^9-tetrahydrocannibol decreased the

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size of the compound action potential of non-myelinated fibres of the vagus nerve of rabbit in concentrations as low as 10 μM it did not affect the Na-K currents in squid giant axon a phenomenon also observed by us for L-*trans*-Δ^8-tetrahydrocannibol in concentrations as great as 0.4 mM (unpublished observations).

Zusammenfassung. Nachweis, dass Lysergsäurediäthylamid-25 (LSD) die Ionenströme von Na und K im Riesenaxon des Tintenfisches auch in hohen Konzentrationen nicht beeinflusst.

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Male Sex Pheromone from the Wing Glands of the Indian Meal Moth, *Plodia interpunctella* (Hbn.) (Lepidoptera: Phycitidae)

Several male phycitid moths including the Indian meal moth, *Plodia interpunctella*, possess a characteristic gland located at the base of each forewing[1]. It consists of a membraneous flap overlying specialized scales that are presumed to release a scent that stimulates the female[1,2] but no function has yet been demonstrated. In the case of the related pyralid moth, *Achroia grisella* (Fabr.), the male wing glands (also located at the base of the forewings) release a sex pheromone that, in combination with sound, attracts the female to the male[3]. However, the male scent scales of other moths and butterflies usually emit a sex pheromone during courtship which acts as an aphrodisiac inhibiting flight and rendering the female receptive to the copulatory attempts of the male[4–7]. This report describes the function of the supposed scent glands of the Indian meal moth.

Observations of mating behaviour were made in a glass petri dish (10 cm diam.). The sexually excited male typically approaches the calling female with his wing glands open; that is, the flap covering the scent scales is raised and the scales themselves are erected and splayed. Generally courtship is initiated when the male approaches from the rear and nudges the underside of the female abdomen and wings with his head and antennae. If receptive, the female turns 180° and usually lowers her abdomen from the calling position. She faces the male head on and remains stationary. The male moves forward and puts his head under the head of the female. The female responds by bringing her antennae forward so that they lie close to the wing glands but apparently do not touch them. Following this, the female raises her abdomen so that it projects between her wings in a position similar to calling but the pheromone gland is not exposed. This is termed the acceptance posture and as soon as it is taken up the male makes genital thrusts and copulation usually follows.

If the female cannot perceive the male scent, however, her courtship behaviour is drastically altered and she rejects the courting male. Elimination of female perception of the male scent was accomplished in two ways, either by bilateral antennectomy of the female or by removing both male forewings including the glands. Controls, to assess the effects of amputation, consisted of females which had about $^3/_4$ of their antennae removed leaving small flagellar stumps which contained numerous chemoreceptors or males which had most (about $^7/_8$) of their forewings removed except the glands which were left attached to the male. All amputations were carried out on insects lightly narcotized with carbon dioxide.

Courtship involving a glandless male and a normal female was initiated in the usual manner. However, the female when nudged on the rear of her abdomen by the male turned either less than or more than 180° so that she did not face the male head on. At the same time she backed up and turned her head and body to one side then the other of the male's head and continued to avoid the head-on configuration with the male. The male advanced as the female backed up and attempted to put his head under hers but her manoeuvers generally prevented this. Moreover, the female did not erect her abdomen between her wings in the typical acceptance posture. Consequently, copulatory thrusts were infrequent during rejection behaviour. Eventually contact was lost between the partners and courtship was terminated. If the male's courtship approach was to

Effect of removal of male wing glands or female antennae on mating success of *Plodia interpunctella* during 10 min observation period

Sex and treatment	No. of pairs	Percent mated
Controls		
♂ and ♀ Normal	92	93
♀ 3/4 Antennaeless	30	83
♂ 7/8 Wingless, gland intact	20	80
Experimentals		
♀ Antennaeless	30	27
♂ Glandless	30	37

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