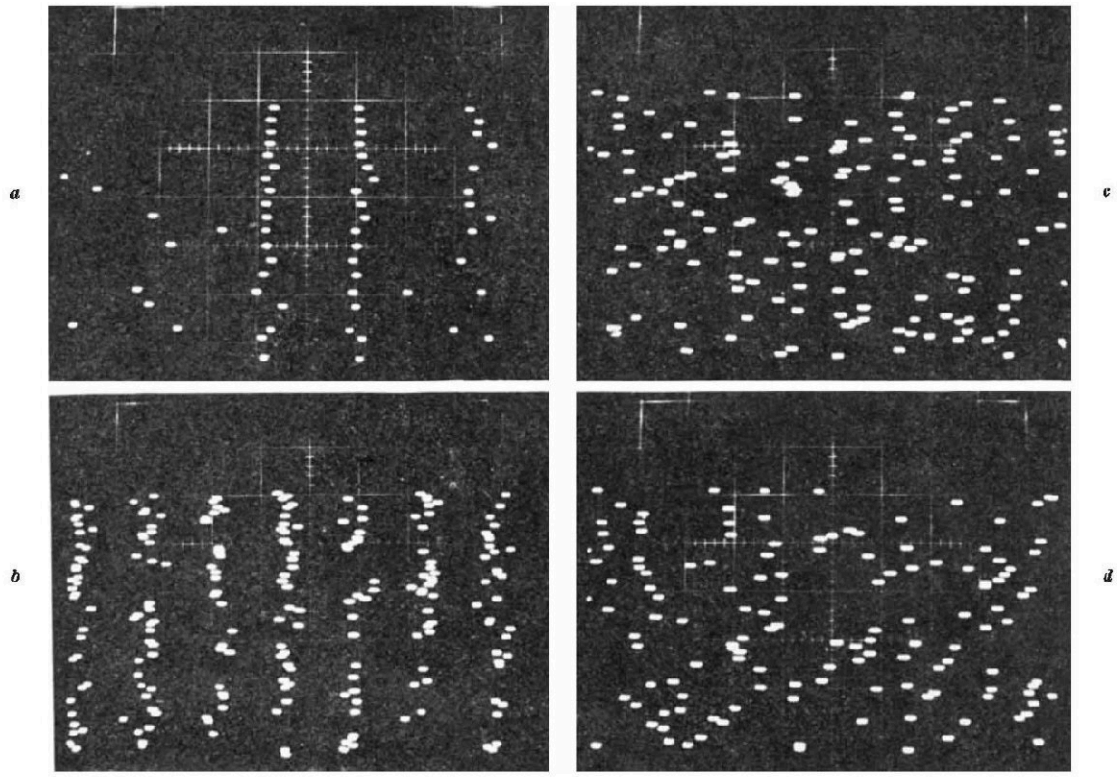

Fig. 1. Raster pattern display of neuronal responses made by shifting the oscilloscope beam vertically after each sweep which is synchronized with a click or a train of clicks. Each dot represents a nerve impulse. Time scale is 1 ms per division. (*a*) Multiple discharges of a single unit in response to a click. The columns of dots are separated by about 2·0 ms corresponding approximately to the reciprocal of the unit's best frequency, 475 Hz. Scattered dots are due to spontaneous firings. (*b*) Near 100 per cent time-locking is indicated by lining up of dots as distinct columns in response to a train of clicks. Note that this unit can follow clicks repeated faster than its best frequency, 1·6 ms versus 2·0 ms. (*c*) Time-locking is visually barely detectable as the interval is reduced to 1·3 ms. (*d*) When the interclick interval is 1 ms the responses are no longer synchronized with the stimuli.

about 1,000 clicks per s. There seems to be a tendency for lower frequency units to resolve shorter intervals, but more data are needed to evaluate the significance of this variation.

In so far as the present sample indicates, the minimum interval resolved by single auditory neurones is rather constant among different species of birds. In comparison with songbirds, a species like the pigeon does not seem to have any rapid sequence of sounds in its vocalizations, yet its auditory neurones can resolve such sounds.

Birds are clearly superior to typical mammals in time resolution. In the cat's primary auditory fibres the degree of time-locking ranges from 100 to 40 per cent at the click repetition rate of 100 per second and drops almost linearly to 10 per cent as the repetition rate approaches 1,000 clicks per second[6]. It is interesting to note that echo locating bats are comparable with birds with respect to time resolution; neurones in the cochlear nucleus of *Myotis lucifugus* show 100 per cent time-locking to a pair of pulses as the interpulse interval approaches 2 ms (ref. 8). The inability of man to hear rapid sequences of sounds in bird songs may indeed be a consequence of the failure of his ears to register such sounds rather than of some central phenomena.

Table 1. TIME RESOLUTION AND BEST FREQUENCIES OF SINGLE AUDITORY NEURONES IN VARIOUS SPECIES OF BIRDS

Minimum interval for nearly 100 per cent time-locking	Best frequency	Species
2·0 ms	4,261 Hz	House sparrow (*Passer domesticus*)
2·5	3,772	" " " "
2·0	1,829	" " " "
1·3	905	" " " "
1·3	733	" " " "
1·6	474	" " " "
2·0	3,952	Song sparrow (*Melospiza melodia*)
1·6	3,500	" " " "
1·3	3,461	" " " "
2·0	2,399	" " " "
1·0	604	" " " "
1·6	4,197	White-crowned sparrow (*Zonotrichia leucophrys*)
0·63	1,591	White-throated sparrow (*Zonotrichia albicollis*)
1·3	604	Pigeon (*Columba livia*)

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MASAKAZU KONISHI

Department of Biology,
Princeton University.

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Comparison of Effects of LSD and Amphetamine on Midbrain Raphe Units

D-Lysergic acid diethylamide (LSD) given parenterally produces a reversible cessation of the spontaneous activity of neuronal units in the midbrain raphe nuclei of the rat[1]. Threshold doses of LSD required to produce this effect are extremely small in relation to those typically used in rats

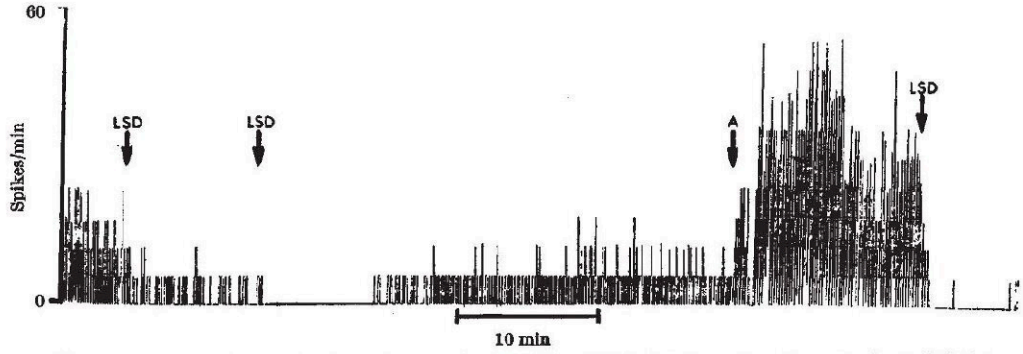

Fig. 1. A record of the activity of a raphe unit after injections of LSD-25 and amphetamine. Each pen deflexion represents a 10 s sample of unit activity. The deflexions are calibrated in terms of "spikes per minute". The first dose of LSD (10 μg/kg, intravenously) slows unit and a second dose of LSD (10 μg/kg) stops unit temporarily. After partial recovery amphetamine (A; 1 mg/kg, intravenously) induces an acceleration of firing which is halted by a third dose of LSD (20 μg/kg).

(10–20 μg/kg, intravenously). Substances the behavioural effects of which are similar to those of LSD (for example, N,N-dimethyltryptamine and mescaline) also inhibit raphe units, although none is as potent as LSD[2]. A variety of other types of drugs (for example, atropine, phencyclidine, chlorpromazine) do not affect the activity of raphe units[2]. The inhibitory effect of LSD is specific for units in the dorsal and median raphe nuclei; in other mid-brain areas (for example, reticular formation), the drug either has no effect or accelerates unit activity[1]. According to the fluorescence histochemical method the midbrain raphe nuclei are comprised of serotonin-containing neurones[3]. Axons of the raphe neurones supply the principal serotonin input to the forebrain[4]. Large doses of LSD induce a prolonged inhibition of the serotonin-containing neurones[1]; this inhibition may account for the reduced metabolism of brain serotonin observed after injections of LSD[5–8].

LSD has certain effects in common with stimulant drugs (for example, EEG activation), and the question arises whether the inhibition of raphe neurones is specific for LSD and related psychotogenic compounds, or whether this effect would generally follow from any sort of stimulant. We investigated this question by comparing the effects on raphe units of LSD and amphetamine, a representative stimulant drug. Amphetamine can be distinguished from LSD behaviourally and neurophysiologically[9], and it does not exhibit cross-tolerance with LSD[10]. Both LSD and amphetamine produce some peripheral adrenergic effects, and the influence of parenteral l-norepinephrine on raphe unit activity was therefore examined.

Seventy-seven male albino rats (Sprague–Dawley strain, Charles River) weighing 250–300 g were anaesthetized with chloral hydrate and placed in a stereotaxic instrument. For the raphe placements a burr hole was drilled at the following coordinates: anterior, 350 microns; lateral, 0 microns[11]. A tungsten microelectrode with a tip diameter of approximately 1 micron was gradually lowered into the dorsal raphe nucleus (that is, midline area, ventral to aqueduct) or median raphe nucleus (that is, midline area ventral to decussation of superior cerebellar peduncle). Electrode pick-up was fed into a high impedence preamplifier and displayed on an oscilloscope. The vertical output from the oscilloscope was led to a rate averaging computer. The output of the computer consisted of an analogue representation of rate and was plotted on a strip chart potentiometric recorder. Typical procedure consisted of locating a unit, observing its spontaneous activity for a period of 5 to 10 min and then giving an initial intravenous dose of either amphetamine or LSD. Raphe units were tentatively identified by their characteristic slow spontaneous rate of firing (~1 spike/s) and regular rhythm[1,2]. Post-injection rate was observed and then an injection of the alternate drug was given. Drugs were given in counterbalanced fashion to avoid possible sequence effects. Body temperature was maintained with an infrared source. After completion of each experiment animals were perfused with saline followed by 5 per cent glutaraldehyde (in 0·15 M sodium phosphate buffer, *p*H 7·4). Serial frozen sections of midbrain were cut and stained with cresyl violet to determine electrode placement.

Of the seventy-seven units studied, thirty-two were in the raphe nuclei. Fourteen of the raphe units responded to injections of d-amphetamine sulphate (0·5–1·0 mg/kg) by a sustained increase to at least double the spontaneous rate. Conversely, these same cells showed a decrease and cessation of firing after LSD; a typical record is presented in Fig. 1. The LSD inhibitory effect was dominant, and units were not accelerated by amphetamine unless they were already partially recovered from the LSD. The remaining eighteen raphe units were also inhibited by LSD but they were relatively insensitive to amphetamine. In the case of these eighteen units, amphetamine produced either a small, transitory increase in rate or had no effect at all. Intravenous injections of l-norepinephrine up to 70 μg/kg did not affect the firing rate of raphe neurones in spite of maximal increases in systolic blood pressure. On the other hand, amphetamine at the doses used (0·5–1·0 mg/kg) did not produce any increase in blood pressure.

In contrast to their disparate effects on raphe units, LSD and amphetamine usually had similar effects on units in the reticular formation and other areas of the midbrain. Of the forty-five non-raphe units studied, eleven showed an increase in rate in response to both LSD and amphetamine. The remaining thirty-four units showed little or no response to either drug.

Histological examination of electrode placement confirmed that the thirty-two units inhibited by LSD were located in either the dorsal or median raphe nuclei. The placements differed with respect to position on the anterior–posterior axis, and generally those units that were accelerated by amphetamine were found in the caudal portion of the dorsal raphe while those not sensitive were found anterior (Fig. 2). Although there does exist some overlap, these data suggest an anatomical sub-differentiation of the raphe nuclei with respect to responsiveness to amphetamine. The fluorescence histochemical technique has demonstrated catecholamine endings close to some perikarya in the raphe nuclei[12]. It is conceivable that the excitatory input for some midbrain raphe neurones originates from catecholamine neurones and that amphetamine acts via this system. Non-catecholamine pathways could also be involved in mediating the amphetamine effect.

We conclude that because amphetamine failed to inhibit raphe units the inhibitory effect of LSD on these units cannot be explained in terms of some non-specific stimulant action. In fact, many raphe units showed opposite responses to LSD and amphetamine (that is, inhibition by LSD and activation by amphetamine). A peripheral adrenergic effect also would not seem to account for the LSD inhibition of raphe units, because even very large parenteral doses of l-norepinephrine had no effect on the firing of these neurones. A drug given

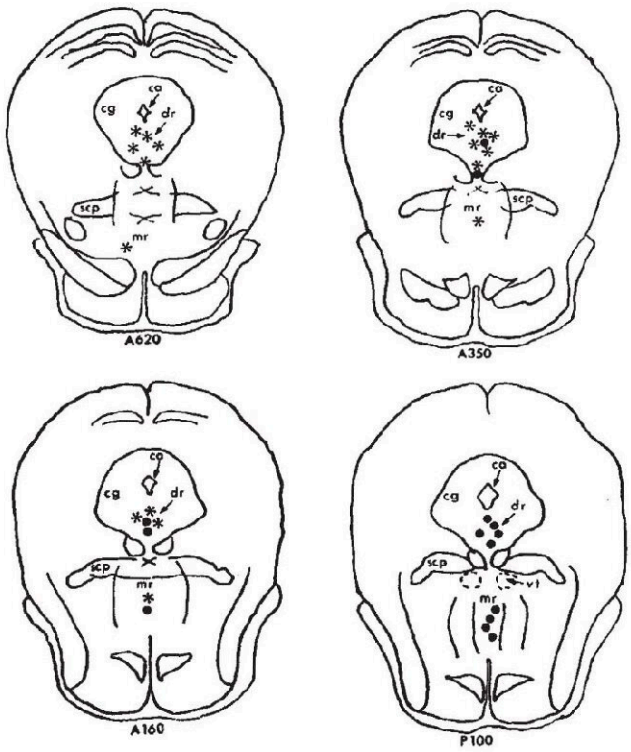

Fig. 2. Summary of electrode placements in the midbrain raphe nuclei. * Sites of units that were either unresponsive to amphetamine or responded with only a slight transient increase in rate; ●, sites of units that responded to amphetamine with at least a doubling of rate. All units were stopped by LSD. *ca*, Cerebral aqueduct; *cg*, central grey; *dr*, dorsal raphe nucleus; *mr*, median raphe nucleus; *scp*, superior cerebellar peduncle; *vt*, ventral tegmental nucleus. Coordinates are based on the atlas of Konig and Klippel[11].

parenterally could act at many sites and LSD might inhibit raphe neurones by a direct or indirect mechanism. It has been suggested that LSD may have a serotonin-like (or mimicking) effect at the post-synaptic sites of the serotonin neuronal system and that this would result in a neuronal feedback inhibition of raphe neurones[1,8,13]. On the other hand, several studies show a blocking of unit responses to serotonin by iontophoretic application of LSD[14–17]. These findings seem to oppose the idea that LSD mimics serotonin at post-synaptic sites. The interpretation of the iontophoretic work is difficult, however, because there is no histochemical evidence that units tested in such studies actually received an input of serotonin fibres. Moreover, as Bradley and Wolstencroft[17] have shown with amphetamine, the effect of a drug applied iontophoretically to a unit may differ from its effect on the same unit after parenteral administration.

WARREN E. FOOTE
MICHAEL H. SHEARD
GEORGE K. AGHAJANIAN

Department of Psychiatry,
Yale University School of Medicine and
The Connecticut Mental Health Center,
New Haven, Connecticut 06508.

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Non-selective Re-innervation of Slow and Fast Muscle Fibres in the Rat

PREVIOUS work[1,2] has indicated that after nerve section the regenerating motor nerve fibres do not preferentially go back to their own muscles. Thus it appears that regeneration of motor fibres is not specific to given muscles, but because muscles are composed of fibres of various types it could still be that regeneration of axons is selective to a given type of muscle fibre. This appears to be the case in the chicken, for Feng, Wu and Yang[3] have reported that after transection of a mixed nerve, containing axons to fast and slow muscles, the nerve fibres selectively re-established connexions with the appropriate muscles. The question of selective regeneration of motor nerve fibres has therefore been re-examined in the rat.

It has been shown previously[4,5] that "fast" and "slow" twitch muscle fibres in the rat differ in their spatial distribution of acetylcholine (ACh) sensitivity. In fibres from a fast muscle, such as the extensor digitorum longus (EDL), the sensitivity to ACh is confined to the vicinity of the neuromuscular junction, while the slow soleus fibres, in addition to a region of high sensitivity at the myoneural junction, show low ACh-sensitivity throughout their entire length.

If the nerve to the fast EDL muscle is transposed to the soleus muscle, replacing its own nerve, the foreign axons establish connexions with the soleus muscle fibres, and the extra-junctional ACh-sensitivity becomes reduced below detectable levels, as is characteristic of fast muscle fibres. This change is not the result of the degeneration and subsequent re-establishment of neuromuscular junctions as such, for if the soleus nerve is cut and allowed to re-innervate its own muscle, the pattern of ACh-sensitivity remains that of normal soleus muscles[6].

These experiments indicate that the spatial distribution of ACh-sensitivity in fast and slow muscle fibres is determined by the type of innervation they receive[6]. Thus they provide us with a means of testing the selectivity of neuromuscular regeneration. For example, the sciatic nerve contains axons normally supplying muscle fibres of different types, in many muscles. If regeneration were type-specific, one would expect that after sciatic nerve section, axons which originally went to slow muscle fibres would re-innervate their own type of fibre, and the normal pattern of ACh-sensitivity should be found. It will now be shown that this prediction is not borne out.

The sciatic nerve of the rat was cut high in the thigh, leaving a gap of a few mm between the cut ends, so as to promote random mixing of nerve fibres during regenerative outgrowth. At least 3 months later, the sensitivity to ionophoretically applied ACh was examined near the myotendinous junctions of the re-innervated soleus muscle, as well as in the control soleus from the opposite side. No ACh-sensitivity could be detected in most of the myotendinous junctions of the re-innervated soleus.