to seasonal differences in the endogenous 5-HT levels. Animals sacrificed at different time period during the same day did not display the large variation demonstrated in the yearly study, but did display a variation of from 5 to 10% of the mean daily value.

In examining the 5-HT levels for the year it now seems quite clear that the levels changed dramatically during the course of the experiment (table). Summer levels of 5-HT were approximately twice those found during the winter. During August and December the catecholamine content was also determined for the same animals in addition to estimating 5-HT levels. During August the DA content was 37.25 ± 0.6 μg/g (mean ± SEM) and NE was 3.57 ± 0.52 μg/g as compared to December when the values dropped to 15.55 ± 0.52 and 2.10 ± 0.11, respectively. These values were found to be statistically significant ($p < 0.005$). The DA and NE levels varied in the same manner as 5-HT, that is high during the summer and low during the winter. The summer values also are approximately twice those found during the winter. In August and December the amount of 5-HT to DA was 1.5 and 1.8, respectively. Since these values remained relatively constant this may suggest an interrelationship between 5-HT and DA as demonstrated for these animals[13].

In M. edulis York and Twarog[9] noted changes in the 5-HT content of the pedal ganglia during March and April (5.4–8.6 to 26–42 μg/g). The present study also demonstrated a sharp increase in the 5-HT levels during this time period. Therefore studies involving monoamine metabolism must be aware of these level changes which may also influence the organisms sensitivity toward endogenous and exogenous agents. These seasonal changes may play a dominant role in the organisms behavior pattern[5]. The reproductive cycle of the animal consists of several seasonal phases: development of resting gonads (fall), gametogenesis (winter), spawning (spring), rapid gametogenesis (early summer) and resting state[14]. The time sequence of the above cycle which is for the English coast can vary from habitate to habitate. In Long Island Sound where our animals were collected the cycle is very similar[15]. Metabolic activity is also seasonal, glycogen accumulates during nonreproductive periods[14] and falls in the winter. Lipids behave in an opposite manner[16]. Studies of environmental factors affecting these cycles are varied and often contradictory. However, temperature changes and neurosecretory activity are known to play important roles[17]. Recently, short-term temperature changes have been shown to alter monoamine metabolism in the CNS of M. edulis[18]. Direct correlation of our findings to the circannual behavior of M. edulis can not be made at this time. However, the regulatory activity of biogenic amines in other organisms would strongly suggest interrelationships. The authors are preparing a more detailed report on factors which may modify these seasonal monoamine changes (temperature, photoperiod and food availability).

Serotonin (μg/g ± SEM) was determined spectrofluorometrically for the CNS (2 cerebral, 2 pedal and 2 visceral ganglia) of M. edulis

Month	N	Serotonin (μg/g ± SEM)
January	8	25.10 ± 2.71
February	4	26.96 ± 2.11
March	4	32.17 ± 3.85
April	4	41.98 ± 1.22*
May	4	48.15 ± 1.02**
June	4	53.13 ± 1.71**
July	4	51.74 ± 3.14**
August	4	57.28 ± 2.49**
September	4	48.90 ± 1.13*
October	4	44.80 ± 1.51*
November	4	35.71 ± 2.70***
December	4	28.97 ± 2.64

N is the number of animals assayed. Significance was determined by a one-tailed Student's t-test. Comparisons for significance were made by comparing January to the rest of the months. * $p < 0.005$, ** $p < 0.001$, *** $p < 0.05$.

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Non-synaptic chemical neurotransmission[1]

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Summary. Images with apparently gemmulofugal polarity in the EPL of the olfactory bulb are the result of sectioning, along misleading planes, gemmulopetal synapses containing postsynaptic vesicles. Unless one accepts a bidirectional conduction for chemical synapses, the internal granule cells lack actual gemmulofugal synapses and the neurotransmitter contained in their vesicles must act at non-synaptic membranes.

It is generally accepted that most synapses permit transneuronal signalling by means of localized chemical reactions leading to specific subsynaptic changes in ionic permeability. But, does this necessarily entail that that chemical neurotransmission should be associated exclusively with the presence of synapses? It has been reported that there is a striking discrepancy between the the large number of synaptic vesicles present in the gemmules of the internal granule cells of the olfactory bulb and the scarcity or, very likely, total absence of gemmulofugal synapses[2]. In this region, gemmulopetal synapses can be easily identified (figure 1, black arrows) because of the selective vesicular accumulation on the side of the mitral or tufted profile. However, many synapses can be seen in the external plexiform layer with clusters of vesicles apposed to both sides of the junction

(figure 2). The possible significance of this pattern has already been discussed[3]. In view of the extreme richness in vesicles displayed by most profiles in electron photomicrographs of the olfactory bulb, the possibility was not excluded that this bilateral accumulation could be due to random and, therefore, devoid of functional significance. In order to assess this interpretation we have now calculated, by means of the binomial distribution, the probability that a specific number of vesicles will accumulate in a given subsynaptic area within a rectangle equivalent to the average bouton profile (figure 3). The dimensions of this rectangle were based on data obtained from the same photographic material presented in a previous communication[2].

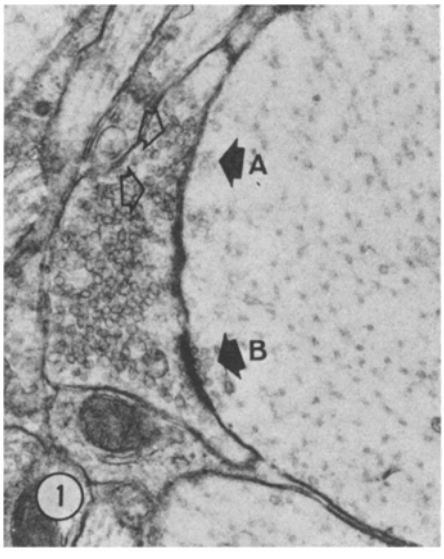

Fig. 1. Region within the external plexiform layer of the olfactory bulb of rabbit. An evident gemmulopetal synapse can be seen in B. It has synaptic vesicles selectively apposed to the junction on the side of the large dendrite (from a tufted or mitral neuron). Open arrows indicate vesicles apposed to the membrane on the gemmule side. The possibility of a gemmulofugal synapse at that level must be ruled out in view of the presence of vesicles on the side of the large dendrite (black arrow in A).

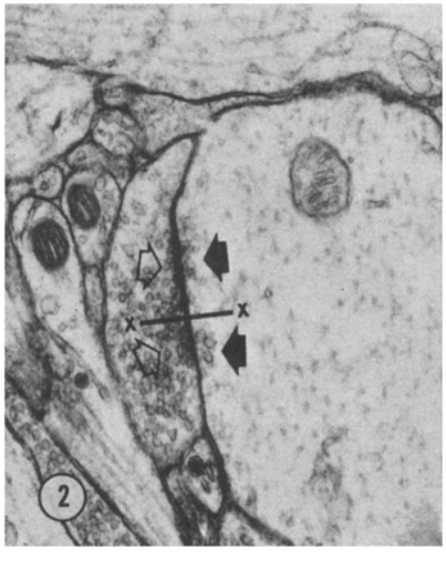

Fig. 2. A synapse with bilateral accumulation of vesicles. In view of the location of the subsynaptic fuzz and the selective accumulation of vesicles on the side of the large dendrite (black arrows), a gemmulopetal polarity must be accepted. Consequently, the vesicles indicated by open arrows are postsynaptic. A section passing along the line x—x would show vesicles only on the gemmule side and convey the erroneous impression of a gemmulofugal polarity.

A total of 60 profiles were analyzed. The average area was 0.310 $\mu m^2 \pm 0.024$ μm^2. Within this area 38 ± 4.6 vesicles were present. The length of membrane apposed to tufted or mitral dendrites was 0.82 ± 0.15 μm. Of this apposed length, a portion was occupied by synaptic specializations in 61% of the boutons. The length of the latter was 0.367 ± 0.18 μm. The probability of a subsynaptic random accumulation of vesicles was then calculated by choosing arbitrarily a subsynaptic area equivalent to $^1/_5$ of the total bouton area. An inspection of figure 2 will show that this is a reasonable specification. In fact, the vesicles of figure 2 accumulate within a relatively smaller area. Therefore, if a given accumulation in the model of figure 3 is regarded as statistically significant, that of the accumulation in figure 2 will be even more significant.

Since the average number of vesicles per bouton was found to be 38, the binomial distribution was computed according to the following formula in which f(x) stands

$$f(x) = \frac{38!}{x!\,(38-x)!}\left(\frac{1}{5}\right)^x\left(1-\frac{1}{5}\right)^{38-x}$$

for the probability that exactly x vesicles will be found in the specified subsynaptic area. From this, it was also possible to calculate the probability of the presence of x vesicles or more (figure 3).

In the particular case illustrated at the top of figure 3, 14 out of 38 vesicles are accumulated in the subsynaptic area. It can be shown that the probability for this number, or more, is less than 2%. In a previous study it was found that 25% of the synapses present in the external plexiform area of the olfactory bulb were comparable to that of figure 2. Therefore, one can conclude that the presence of subsynaptic vesicular accumulations within the granule cell gemmules is statistically significant. This reinforces the conclusion reached at that time that the presence of 'return' synapses is questionable. Until proof to the contrary, these junctions must be interpreted as an ordinary gemmulopetal synapses cut along misleading planes. The gemmules are so rich in vesicles that any investigator may produce a biased sample and accumulate photographs of apparently gemmulofugal synapses if he is given enough time to choose systematically those junctions with postsynaptic vesicles in which the presynaptic ones are accidentally scarce. For example, in figure 2, a section passing through the line x–x would convey the image of an apparently gemmulofugal polarity.

In view of the above, we have previously expressed the opinion that the internal granule cells of the olfactory bulb lack gemmulofugal synapses and that the transmitter contained in their vesicles (very likely GABA) must be able to act at non-synaptic membranes. This should not be regarded as a revolutionary conclusion. It is implied in a number of well established facts. For example, GABA was found to have an inhibitory action in the 6th abdominal segment of the crayfish, which lacks inhibitory innervation[4]. It should be noted that GABA depolarizes

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the posterior root ganglia[5,6] and conscientious electron microscopic studies in several species have failed to demonstrate the presence of synapses in the perikarya of primary sensory neurons[7,8]. A non-synaptic, gabaergic neurotransmission could account for the fact that GABA-T is not selectively located in the subsynaptic region[9] (in contrast to GAD, which displays a highly selective parajunctional distribution[9,10]).

It has been long known that the smooth musculature of viscera is innervated by nerve fibers which lie merely embedded in sarcolemma gutters within which synapse-like contacts are very rarely seen[11,12]. The same applies to vessels[13,14], to vas deferens[15] and to glandular parenchima[16,17]. No morphologically identifiable specializations have been demonstrated as a basis for the various adrenergic alpha and beta receptors. Certain presynaptic (extrajunctional) regions in motor nerve endings appear to be sensitive to acetylcholine[18] and it seems that this transmitter is capable of triggering antidromic discharges in motoneurons even in the absence of mioneural junctions[19]. An equally presynaptic action for ACh has been claimed in the hippocampus[20]. In denervation supersensitivity, ACh receptors appear in extrajunctional regions of the membrane[21]. In the CNS cholinergic mechanisms are well-documented[22,23]. However, AChE has often been located in regions like the granular endoplasmic reticulum well removed from evident synaptic specializations[24]. A submembranous localization of AChE has also been described within the CNS[25]. But the extent of membrane displaying richness in enzyme appears much greater than can be accounted for by the the presence of synaptic specializations. Serotonin endings in the subependymal layers of the cerebral ventricles do not appear to be associated with evident junctional specializations[26,27]. Nerve endings presumably rich in biogenic amines have been identified in the striatum which appear to be surprisingly poor in synapses[28–30]. Descarries et al.[31] have emphasized that the serotonergic nerve varicosities of rat neocortex display a very low incidence of evident synaptic specializations. A non-synaptic aminergic neurotransmission could account for the fact that the distribution of MAO seems to be confined to mitochondria[32] which are present in nerve endings but not selectively confined to subsynaptic regions. Numerous receptors have been recently isolated in various parts of the nervous system on the basis of their specific binding properties. In view of the above, one may wonder if they may often be associated with non-synaptic regions of the nerve membrane.

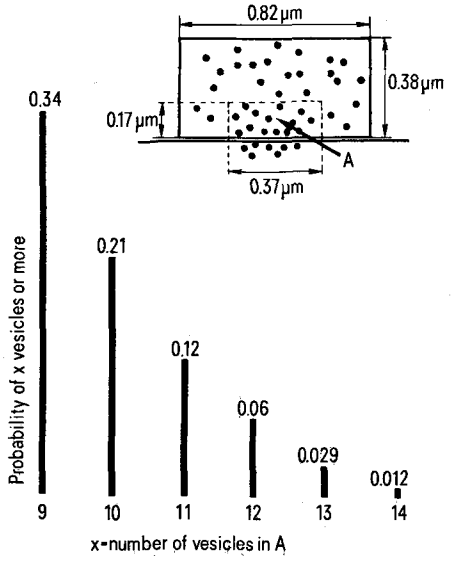

Fig. 3. The average bouton profile in the external plexiform layer of the olfactory bulb of rabbit can be regarded as equivalent to a rectangle with the dimensions specified at the top. Within this rectangle, compartment A occupies $^1/_6$ of the total bouton area. The probability that out of 38 vesicles x, or more, will be found in compartment A is illustrated by the vertical bars.

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