All the above peptides show very little affinity for δ receptors (Table 1). None of them has an IC_{50} value below 10 μ*M* in inhibiting the binding of ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin to rat brain membrane preparations.

Kosterlitz and colleagues have provided strong evidence that the myenteric plexus of the guinea pig ileum contains mainly μ receptors and the mouse vas deferens mainly δ receptors (*1, 15*). Opiates and enkephalins suppress the electrically stimulated smooth muscle contractions of these two isolated organ preparations by inhibiting the release of excitatory neurotransmitters. Morphine is more potent than enkephalin in inhibiting the muscle contractions of the guinea pig ileum, whereas the reverse is found in the mouse vas deferens. Morphiceptin has a potency comparable to morphine in inhibiting electrically stimulated contractions of myenteric plexus–longitudinal muscle preparations (median effective dose, $ED_{50} = 1.3 \times 10^{-7} M$), whereas concentrations 100 times higher are required for depression of the mouse vas deferens ($ED_{50} = 1.5 \times 10^{-5}$, which is about 15 times higher than that of morphine). Naloxone reversed or blocked the depressant effects of morphiceptin in both preparations. Morphiceptin is without effect on electrically stimulated contractions of the rat vas deferens at concentrations as high as $10^{-5} M$. Other casomorphin analogs and fragments show considerably less activity (see Table 1). The ranking of potencies of the casomorphin analogs and fragments in the ileum bioassay are in excellent agreement with their binding affinities to μ receptors in brain membrane preparations. These data substantiate the highly selective affinity of morphiceptin for μ receptors.

Although opioid alkaloids and enkephalins show selectivity for μ and δ receptors, the significant cross-reactivity between receptor subtypes has make it difficult to understand the mode of action of opiates and the selective physiological role of the opiate receptor subtypes. The availability of a relatively specific ligand for the μ receptor subtype should be especially useful in this respect.

The discovery of this specific μ receptor ligand supports the hypothesis of multiple opiate receptors, and the analgesic property of morphiceptin (*14*) clearly establishes the role of μ receptors in modulating pain perception. The possibility of designing a morphine receptor-selective drug with less unwanted side effects may now be feasible. The highly peptidase-resistant property of morphiceptin (*13, 16*) is also attractive. The physiological significance of this and related peptides in milk remains to be determined.

A peptide isolated from enzymatic digests of β-casein is very similar or identical to morphiceptin (*17*). It behaves similarly on high-pressure liquid chromatography and gel filtration, and it cross-reacts with antibodies selective for morphiceptin that react only poorly with the carboxyl-free tetrapeptide. Perhaps this or related peptides derived from dietary constituents are able to interact physiologically with μ receptors under certain circumstances.

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12. β-Casomorphin and its fragments were synthesized by a solid-phase method. After hydrogen fluoride cleavage of protected peptide from the resin, the amide peptide was purified by counter-current distribution with the use of *n*-butyl alcohol, acetic acid, and water [*n*-BuOH: HOAC:H_2O (4:1:5 by volume)] as partition solvents. Peptides were characterized by thin-layer chromatography, electrophoresis, amino acid analyses, and high-performance chromatography. The purity of the peptide was 98 percent or better.
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Genetic Basis of Migratory Behavior in European Warblers

Abstract. *The seasonal course and magnitude of migratory restlessness recorded in four populations of the blackcap* Sylvia atricapilla *differ in a population-specific fashion that is related to the distance of travel. Experimentally produced hybrids of an exclusively migratory European population and a partially migratory African population showed intermediate migratory restlessness and an intermediate percentage of birds displaying restlessness compared to the two parental stocks. These results demonstrate the genetic basis of migratory behavior in this avian species and support the hypothesis that partial migration of populations is due to polymorphism.*

In Old World warblers of the genera *Phylloscopus* and *Sylvia* there are close relations between migratory restlessness (nocturnal hopping by captive birds at the time of migration) and several aspects of actual migration. In particular, the amount of migratory restlessness measured in the laboratory is closely correlated with the length of the migratory route. Thus it is possible that young, inexperienced birds migrate to their species- or population-specific winter quarters as a result of an endogenous program for migration that is controlled by or linked with an endogenous annual rhythm (*1*).

These observations can be explained by assuming that the patterns of migratory behavior are genetically fixed (*2*). Alternatively, environmental conditions (such as differences in photoperiod, temperature, or food supply) under which individuals grow up may be responsible for the differences in migratory performance (*3*). The genetic hypothesis would be supported if F_1 hybrids of birds from two populations that migrate over different distances showed intermediate patterns of migratory behavior (*4*).

The blackcap *Sylvia atricapilla*, which has a wide distribution from the Cape Verde Islands off the West African coast to approximately 65°N in Europe, is a suitable species for the necessary cross-breeding experiment. Blackcaps of different populations differ sharply in their migratory performance and in the percentage of individuals that display migratory restlessness when caged. Moreover, migratory activity can be easily measured as nocturnal activity in this nocturnally migrating species (*5*).

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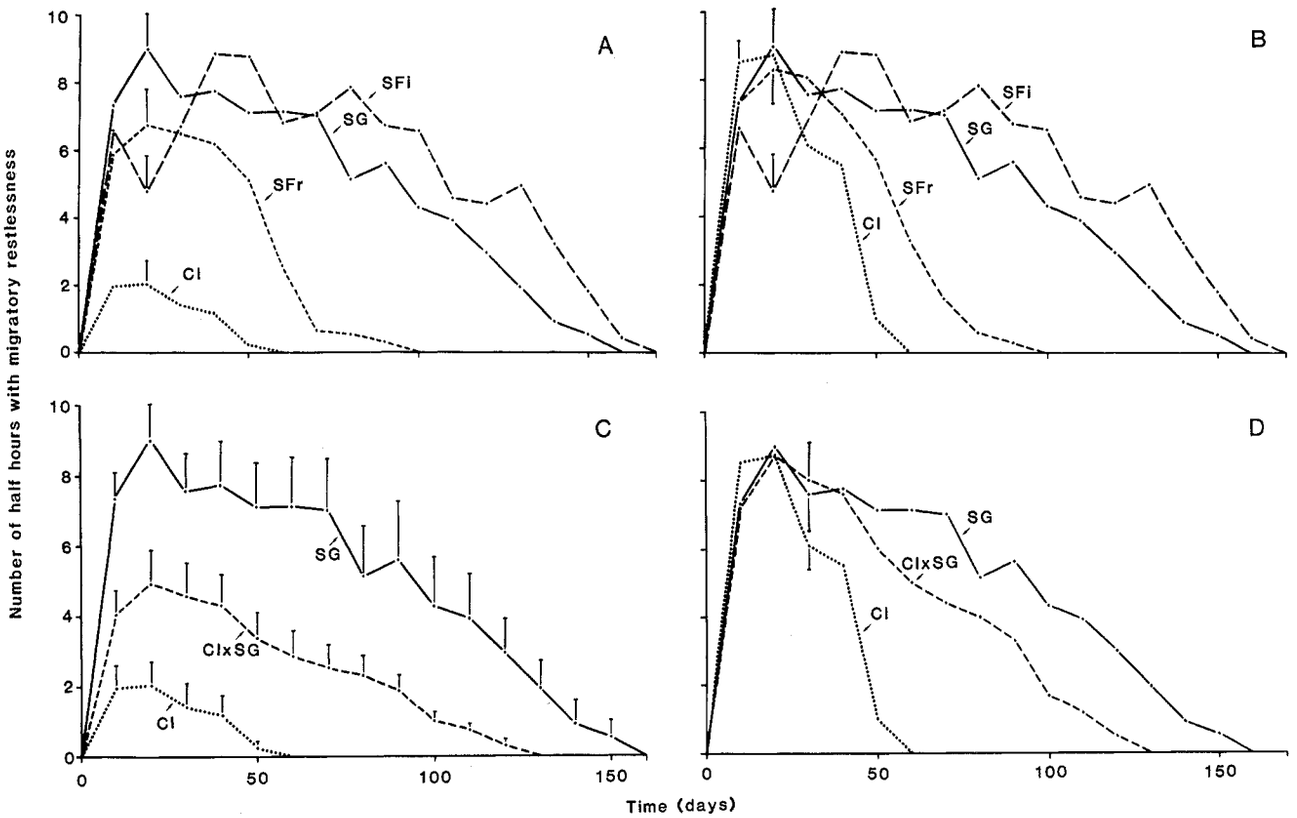

Fig. 1. (A and B) Time course of nocturnal migratory restlessness of groups of *Sylvia atricapilla* from three European and one African populations. Mean values for 10-day periods are given. Abbreviations for sources of the birds; *SFi*, southern Finland (60°N); *SG*, southern Germany (47°N); *SFr*, southern France (43°N); and *CI*, Canary Islands (28°N). (A) Data for all the birds in each group, including those birds that did not show migratory restlessness (N = 26, 25, 24, and 26 for *SFi*, *SG*, *SFr*, and *CI* birds, respectively). Significant differences between groups are as follows (Mann-Whitney U test): for *SFi* birds compared to *SFr* and *CI* birds, $P < .001$; for *SFr* birds compared to *SG* birds, $P < .01$; and for *SG* birds compared to *CI* birds, $P < .001$. (B) Data for the birds that showed restlessness (N = 26, 25, 20, and 6). All curves are normalized so that they start on the first night of restlessness for each bird. To maintain clarity, standard errors are given for representative examples. (C and D) Time course of migratory restlessness of hybrids (*CI* × *SG*) compared with that of the *SG* and *CI* parental stocks. (C) Data for all the birds in each group, including those birds that did not show migratory restlessness (N = 25, 32, and 26 for *SG*, *CI* × *SG*, and *CI* birds, respectively). Standard errors are given for all the mean values. (D) Data for the birds that showed migratory restlessness (N = 25, 18, and 6); standard errors are given for representative examples.

Twenty-four to 26 nestlings from each of three European populations (Finnish, German, and French) and one African population were hand-raised in the spring of 1976 and 1977 and their migratory restlessness was recorded in the following autumns (Fig. 1, A and B). The degree of migratory restlessness was greatest in the Finnish birds, with progressively less in the German, French, and African birds. One hundred percent of the Finnish and German birds, 80 percent of the French birds, and 23 percent of the African birds exhibited restlessness [$P < .001$ for the African birds compared to the Finnish and German birds (chi-square test)]. These results are in accordance with the distances of north-south travel in the free-living populations (*6*).

In 1978 and 1979 we used the African and German birds of the above experiment for a cross-breeding experiment. We successfully hand-raised 32 hybrids and used the same method to record their migratory restlessness as had been used for their parents. Twenty-eight of the 32 hybrids were derived by crossing migratory German and nonmigratory African birds; four were derived from a migratory German parent and a migratory African parent.

The hybrids displayed an amount of migratory restlessness intermediate between that of the parental populations of Africa ($P < .001$; Mann-Whitney U test) and Germany ($P < .01$) (Fig. 1, C and D). Also, the mean number of restless nights, the range of the amount of restlessness, and the coefficient of variation for restlessness were all intermediate in the hybrids (*7*).

There is consistent agreement between the proportion of migrants and nonmigrants in various blackcap populations and the proportion of birds in those populations which display migratory restlessness in captivity. Therefore the migratory or sedentary habits of different individuals constituting the partially migratory populations of blackcaps may be due to polymorphism (*5*). In our cross-breeding experiment all the German parental birds and 23 percent of the African ones displayed migratory restlessness. If polymorphism is the basis of the partially migratory behavior, the introduction of genes from the exclusively migratory German population into the partially migratory African population should increase the percentage of F_1 hybrids displaying restlessness. We observed restlessness in 56 percent of the hybrids. This value is intermediate between, and significantly different from, those obtained for the German parental population ($P < .001$, chi-square test) and the African parental population ($P < .025$). In addition, the expression of morphological features and juvenile development associated with migration was also intermediate in the hybrids (*8*).

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6. The birds were taken from their nests at 5 to 7 days of age, transferred to the Vogelwarte Radolfzell in southern Germany (47°46'N, 09°00'E), and hand-raised under simulated local light conditions standardized according to an arbitrary birth date of 10 May for all birds. Beginning at 50 days of age the birds were maintained under constant conditions [a cycle of 12.5 hours of light (400 lux) and 11.5 hours of darkness (0.01 lux); 20° ± 1.5°C] until the end of the season of migratory restlessness. Then they were transferred to natural southern German light conditions to allow them to achieve their normal spring breeding condition.
7. The German, F_1 hybrid, and African birds averaged 74, 53, and 35 nights of restlessness, respectively, and displayed restlessness during 0 to 2009, 0 to 991, and 0 to 534 total half-hours. Respective coefficients of variation: 145, 112, and 98 percent.
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9. We thank G. J. Kenagy for his especially extensive review of our report and the Finnish, French, Spanish, and German governments for permission to collect the birds.

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Selection of a Novel Connection by Adult Molluscan Neurons

Abstract. *Predictable changes in neuronal connectivity can be induced in the buccal ganglia of adult* Helisoma *snails when neuritic growth is evoked by axotomy. Both transient and stable novel electrical connections are established between identified neurons. The breaking of inappropriate, normally transient connections is contingent on the formation of an appropriate connection.*

The establishment and maintenance of specific connections in the nervous system is the culmination of a sequence of as yet poorly defined processes. In principle, the selection of such connections may depend on the initial formation of contacts with a range of possible targets, appropriate connections being maintained and inappropriate ones broken. We report here the establishment of both transient ("inappropriate") and stable ("appropriate") novel electrical connections between identified, adult neurons of the snail *Helisoma*. The mechanism accounting for the transient nature of some connections is of fundamental importance. In this system, the breaking of certain inappropriate connections requires the formation of an appropriate connection. Thus, specific patterns of connectivity are determined by the availability of potential targets.

Our initial studies (*1*) of the dynamic abilities of adult molluscan neurons demonstrated that neuritic growth is initiated within a few hours after axotomy. We now report that such outgrowth can result in the selective formation of a new, stable electrical connection between a pair of homologous buccal ganglion neurons, L5 and R5. This pair of neurons, in contrast to many homologues of gastropod buccal ganglia (*2*), are not normally electrically coupled (Fig. 1A). Furthermore, the normal neurites of each neuron 5 are restricted to the ipsilateral ganglion of the paired buccal ganglia (Fig. 1B).

Isolated, paired buccal ganglia of the pulmonate mollusk *Helisoma trivolvis* were cultured in host snails (*1*). Both esophageal trunks were crushed within 100 μm of their origins in order to evoke neuritic growth in the neuropil (*1*). Intracellular stimulation was performed with a balanced bridge circuit and intracellular recordings were made with 2*M* potassium acetate-filled electrodes. The neurons were stained with Lucifer Yellow CH and viewed as whole mounts (*1*, *3*).

The onset of novel coupling between the homologues of neuron 5 (Fig. 1C) was both rapid and predictable; the new connection has characteristics of a direct electrotonic synapse (*4*). Coupling between the homologues of neuron 5 was detected in eight of nine preparations after 2 days of culture and in all 38 after 5 to 30 days (Fig. 2A). The magnitude of coupling was maintained for at least 1 month; coupling coefficients at 7 days and 25 to 30 days were 0.17 ± 0.05 (N = 10) and 0.16 ± 0.04 (N = 7), respectively (*5*). In addition, although recordings become more difficult in older preparations (*6*), coupling of neurons L5 and R5 was observed in all three ganglia successfully recorded after 3 months of culture. That the 5-5 connection is direct and does not involve intervening neurons was further supported by morphological studies demonstrating that neurites from each neuron 5 rapidly traverse the commissure and project toward the contralateral homologue (Fig. 1D). Such newly formed neurites were observed in each of 13 preparations in which coupling was detected and subsequent dye fills made. This absolute correlation of morphology with function supports the role of the newly formed neurites as the substrate of the novel connection and mitigates against the possibility that axotomy is merely revealing a latent, weak connection. In addition, neuritic sprouting across the buccal commissure was observed in two of seven preparations maintained for 24 hours in physiological saline. The speed and inevitability with which such changes occur in *Helisoma* provide a general caution regarding the use of isolated neural preparations for physiological analysis.

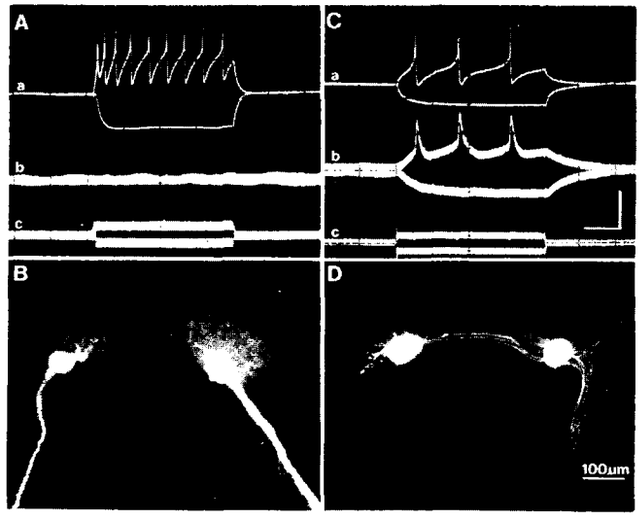

Fig. 1. Physiology and morphology of the electrical coupling between the homologues of neuron 5. (A) Lack of electrical coupling between the homologues of neuron 5 in a normal pair of buccal ganglia. Passage of depolarizing and hyperpolarizing currents (*c*) into L5 (*a*) does not elicit voltage deflections in R5 (*b*); two sweeps are superimposed. (B) Normal morphology of neurons L5 and R5 as shown by staining another preparation with Lucifer Yellow CH. The axon of each neuron 5 exits via an ipsilateral nerve (the esophageal trunk) and the dendrites do not cross the commissure between the paired buccal ganglia. (C) Electrical coupling between the neuron 5 homologues in a preparation cultured for 5 days. In this example the coupling coefficient is 0.22 and action potentials evoked in L5 (*a*) evoke electrotonic excitatory postsynaptic potentials in R5 (*b*). (D) Morphology of neurons L5 and R5 in a preparation cultured for 7 days. The axons of both neurons have been resorbed and newly formed neurites are visible traversing the buccal commissure and in a number of nerve trunks. Calibration for (A) and (C) is 200 msec (horizontal), 25 mV (upper traces), 5 mV (middle traces), and 2 nA (lower traces).

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