females of Ll × O cross, however, showed a significantly higher bias towards homogamic males than did 9-day-old virgins.

Prior copulatory experience affected subsequent mating choices made by *Drosophila* females; the direction of the change depended on whether the crosses were within a species, between semi species or between sibling species. We showed previously[1-3] that following a heterogamic copulatory experience *D. pseudoobscura* females showed a strong bias towards males of the same karyotype as their initial mate. *D. paulistorum* females which mated with a homogamic male showed an increased tendency to remate with the same type males. Comparison between proportion of homogamic matings of sexually experienced and virgin 9-day-old females were statistically significant for all crosses tested. No effect of this magnitude due to initial heterogamic mating experience was observed. *D. melanogaster* and *D. simulans* females, however, showed a significant reduction in ethological isolation after both homo- and heterogamic initial copulatory experience, and in fact, choices made by females with heterogamic mating experience did not differ significantly from random mating. When *D. pseudoobscura* is similarly pitted versus its sibling species *D. persimilis*, Spieth found that each of these closely related species responds differently[11]. The females of both species will immediately accept their own male as soon as they are able to, even though under duress they had accepted the male of the other species and a *D. persimilis* female (like *D. paulistorum*), once she has been inseminated by a *D. persimilis* male, will never again accept a *D. pseudoobscura* male. The *D. pseudoobscura* female is never so restricted and is simply always less sexually isolated from potential *D. persimilis* mates (as *D. melanogaster* - *D. simulans*).

It is not immediately apparent why an initial 'no choice' mating experience had such diverse effects on subsequent choices of mates in these drosophilids. One may speculate that within a species, i.e., with *D. pseudoobscura*, selective pressures exist to enhance outbreeding via heterogamy, and at least an initial mating with a less frequent male will serve to increase the frequency of his karyo- or genotype in the population. For species in statu nascendi however, such pressures must be directed toward the avoidance of less fertile heterogamic matings.

Drosophila mating behavior has been viewed as primarily genetically determined and barely modifiable by experiences during development[12]. The results of these studies show, however, that previous copulatory experiences alter subsequent choice of mates and indicate that sexual isolation contains an acquired component.

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Regional and strain variation in brain 3′:5′ cyclic adenosine monophosphate of inbred mice

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Summary. Regional variations were found in cAMP levels in flash frozen mouse brains with the pons and cerebellum having higher levels than the cerebrum. There were also strain variations with CBA/J and BALB/cJ having higher levels than C57B1/6J in the pons and cerebellum.

It is generally accepted that the binding of neurotransmitter stimulates cyclic AMP (cAMP) synthesis in some receiving cells[1]. There have been multiple attempts to study cAMP levels in brain samples[2,3] and several investigators have reported variations in brain cAMP levels among inbred strains of mice that may correlate with strain variations in behavioral parameters[4-6]. This work has neglected regional differences and indicated surprisingly high levels. In some of this work microwave irradiation was used to kill the mice and cAMP was measured for whole brain and expressed in terms of wet weight. This microwave method of killing the animal is not optimal[7]. We have re-investigated cAMP levels in several strains in a standardized method using liquid nitrogen to inactivate cAMP-degradative activity and have expanded the measurements to four regions of the brain.

Materials and methods. Male mice (6-week-old or older) were killed by decapitation such that the heads fell directly into 5 cm or more of liquid nitrogen. The procedure was standardized so that no more than 35 sec. elapsed from the moment the mouse was first disturbed until the brain was frozen solid (between 10 and 20 sec. elapsed from opening the cage until the head was submerged in liquid nitrogen). More important, in order to minimize the effects of ischemia[3], no more than 10 sec elapsed from the instant of decapitation until total inactivation of all neural enzymes[8]. The brains were then removed from the liquid nitrogen and immediately placed on dry ice and transferred to a cold room for dissection. Working in a cold room on dry ice, the head was bisected revealing a midsagittal view. 4 regional samples, about 1 × 3 mm and approximately 10 mg in wt of upper cerebrum, lower cerebrum, pons, and cerebellum of the brain were taken. Each frozen brain sample was placed in a small test tube for storage. 400 µl of warm (70 °C) dH_2O were pipetted into each tube containing the frozen tissue sample and immersed in a boiling water bath for 13 min. Samples were then removed and centrifuged for 20 min at 2400 × g at 4 °C. 100 µl of each supernatant (in

Table 1. Brain cAMP levels in pmoles cAMP per mg protein

Strain	Upper cerebrum Mean SE	N		Lower cerebrum Mean SE	N		Pons Mean SE	N	Cerebellum Mean SE	N
CBA/J	1.75±0.36	4	t=2.78	1.76±0.27	5	t=2.23	1.92±0.40	5	2.41±0.61	4
C57B1/6J	0.59±0.24	7	p<0.05	0.71±0.33	8	p<0.05	1.13±0.41	6	1.13±0.34	7
A/J	1.34±0.38	5		1.03±0.22	5		1.19±0.44	5	1.85±0.18	5
BALB/cJ	1.09±0.42	9		1.27±0.40	8		2.41±0.47	9	2.07±0.35	9

Each strain was compared to the other 3 across all 4 regions using Student's t-test. Significant differences are indicated by the brackets.

Table 2. Intra-strain comparisons between cerebral region and pons-cerebellum region for cAMP in pmoles per mg protein

Strain	Cerebral region Mean cAMP level SE	N	Pons-cerebellum region Mean cAMP level SE		N	t-test for regional differences
CBA/J	1.76±0.20	9	2.14±0.34	t=2.46 p<0.05	9	0.97, n.s.
C57B1/6J	0.65±0.20	15	1.13±0.25	t=2.80 p<0.01	13	1.49, n.s.
BALB/cJ	1.17±0.28	17	2.24±0.29		18	2.66, p<0.02
A/J	1.18±0.21	10	1.52±0.25		10	1.04, n.s.

In addition to the t-test for regional differences, each strain was compared to the other 3 for the 2 summed regions using Student's t-test. Significant differences are indicated by the brackets.

duplicate) were aliquoted for the cAMP assay and the remaining liquid and pellet were saved for protein determinations by the method of Lowry. Samples were preserved at −70 °C whenever necessary. cAMP was assayed by a modification[9] of the competitive-binding protein assay of Gilman[10] using a commercially available kit (Amersham) and results are expressed in pmoles cAMP per mg of brain protein. The ability of the assay to measure the small levels of cAMP present in samples was confirmed by adding known amounts of cAMP (1-4 pmoles) to duplicate aliquotes of previously measured samples. We confirmed that the reactive substance was cAMP because reactivity was destroyed by treatments with 3′:5′ cyclic nucleotide phosphodiesterase (10 µg/100 µl sample of 0.21 units/mg Sigma Chemical Co., PO 134).

Results and discussion. Mean cAMP levels were determined in the 4 brain regions for each of the 4 strains (table 1). Statistically significant strain variations were found between the C57B1/6J and CBA strain for the upper and the lower cerebral regions. In both cases CBA was higher than C57B1/6J ($p < 0.05$, by Student's t-test). Although no other significant strain variations were observed in the 4 individual regions, A/J and BALB/cJ were intermediate between CBA and C57B1/6J in samples of cerebrum, while BALB/cJ and CBA/J tended to be higher than the others in the sample of pons and cerebellum. Since the 2 cerebral samples and the pons and cerebellum samples were not significantly different in all cases per strain, they were pooled for comparison (table 2). For the BALB/cJ strain, the pons-cerebellum cAMP level was significantly higher than the cerebrum region ($p < 0.02$). The other 3 strains did not show statistically significant regional variation. Using the pooled data (table 2), additional strain variations were found for the pons-cerebellum region between BALB/cJ and C57B1/6J and between CBA and C57B1/6J. In both cases C57B1/6J was lower than BALB/cJ and CBA ($p < 0.01$, $p < 0.05$, respectively).

The levels of brain cAMP we obtained are in the range of those reported by Steiner[3] but low compared to those reported by others[4-6]. This may be explained by the rapid freezing method employed coupled with the minimal animal handling time. It is probable that this more rapid freezing method provides a better approximation to actual in vivo levels of cAMP than some microwave techniques which may elevate levels[7,8]. Although BALB/cJ was the only strain in which the pons-cerebellum showed a statistically higher cyclic AMP level than the cerebrum, a similar trend was observed in the other 3 strains. Schmidt[2] found, using microwave irradiation of rat brains, a similar regional variation in cAMP. This is in contrast to Steiner's[3] findings, in which mouse cerebral cortex was found to be higher than cerebellum, although the difference was small. Our data suggest a rank ordering of strains for cerebral cAMP levels of CBA > BALB/cJ ≅ A/J > C57B1/6J and an order of BALB/cJ ≅ CBA > A/J > C57B1/6J for pons-cerebellum. This does not agree with the ordering obtained from Stalvey's[6] data on cerebral slices: A/J > CBA > C57B1/6J. Orenberg[5] reported a strain variation for whole brain cyclic AMP of C57B1/6J > BALB/cJ > CBA > A/J, with a significant difference between BALB/cJ and CBA. These differences clearly point to the need for standardization of techniques across laboratories to resolve questions of regional and strain variations in brain levels of cAMP.

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