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20. Possible between-task differences in saccadic scanning raises the qustion of artifactual origin of the results. Several considerations counterindicate this: (i) Trials with eye movements visible in the electrooculogram (EOG) were eliminated; averaged EOG's from trials used revealed no consistent differences between the tasks. (ii) Contamination due to potentials generated by eye movements should be strongest in the frontal regions, whereas the patterns extracted by pattern recognition were varied and included the whole scalp. Frontal differentiation was not the most accurate (parietal differentiation during the P3b interval was most accurate), and it was not significant during the N1-P2 interval, which is just after the minimum time required to produce a saccade. And (iii) the presence of spatially differentiated patterns during the cued prestimulus interval and the lack of differences just prior to response support the interpretation of neural genesis of the results.

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DDT-Induced Feminization of Gull Embryos

Abstract. *Injection of DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] into gull eggs at concentrations comparable to those found in contaminated seabird eggs in 1970 induces abnormal development of ovarian tissue and oviducts in male embryos. Developmental feminization of males is associated with inability to breed as adults and may explain the highly skewed sex ratio and reduced number of male gulls breeding on Santa Barbara Island in southern California.*

Between 1950 and 1970 offshore southern California was subjected to massive contamination by the discharge of as much as 1.9 million kilograms of commercial DDT (*1*) from the Los Angeles sewer system (*2, 3*). This resulted in high residues of total DDT (*4*) at all levels of the ecosystem, with particularly high concentrations in fish, sea lions, and seabirds (*5*). The breeding failure of brown pelicans (*Pelecanus occidentalis*) and double-crested cormorants (*Phalacrocorax auritus*) due to eggshell thinning has been well documented (*6–8*). High concentrations were measured in eggs of western gulls (*Larus occidentalis*) 3 years after sewer discharge of DDT ceased (*9*).

We report that DDT contamination of eggs during the early 1970's may be involved in the current reproductive failure of western gulls in the Channel Islands. The poor breeding success is characterized by a reduced number of adult males, a highly skewed sex ratio (3.85 females for each male on Santa Barbara Island), and female-female pairing of some of the excess females (*10*). We propose that DDT contamination of eggs and embryos causes abnormal development of the reproductive system and results in breeding failure in the adult birds.

Gull eggs are relatively insensitive to the thinning effects of DDT (*11*). Concentrations that result in severely thinned and broken pelican eggs (40 to 80 ppm per whole egg, by weight) (*12*) result in only modest (6 to 10 percent) thinning in gull eggshells; embryos survive until concentrations reach 200 to 300 ppm (*13*). However, this resistance to eggshell thinning puts gull embryos at greater risk of exposure to the estrogenic effects of DDT metabolites. We exposed gull embryos to DDT at historical levels (2 to 100 ppm) to determine the effects that probably occurred in the wild.

Eggs were collected in 1979 and 1980 from breeding colonies at Mono Lake (California gulls, *Larus californicus*) and Southeast Farallon Island (western gulls). These places do not have a history of high DDT contamination. An attempt was made to obtain unincubated eggs by collecting the first egg laid in a nest at the beginning of the nesting season. Recrystallized pesticides and metabolites or estradiol were dissolved in corn oil and injected directly into the yolk (*14*). This method closely approximates the distribution of the pollutants in eggs in the wild, since these compounds are lipid-soluble and accumulate in yolk. The injected pollutants do not immediately mix with yolk, but become mixed as the yolk is mobilized during the first few days of incubation. Injected eggs were incubated artificially (*15*), and embryos that survived to hatching were fixed and examined.

Two hundred sixty-four eggs were successfully injected, and 108 developed to pipping, the point at which the embryo is ready to cut the shell with its egg tooth. Many embryos died (45 percent died before they had been incubated for 2 days), probably because of transport, storage, and injection. Data from both species were pooled since no differences were observed in sensitivity to the compounds or extent of developmental abnormalities.

The anatomy of gulls at hatching and their response to estradiol or estrogenic compounds resemble those of chickens and Japanese quail (*16, 17*), but gulls are much more sensitive to the effects of estrogens. All male embryos at all doses of estradiol were feminized. The extent

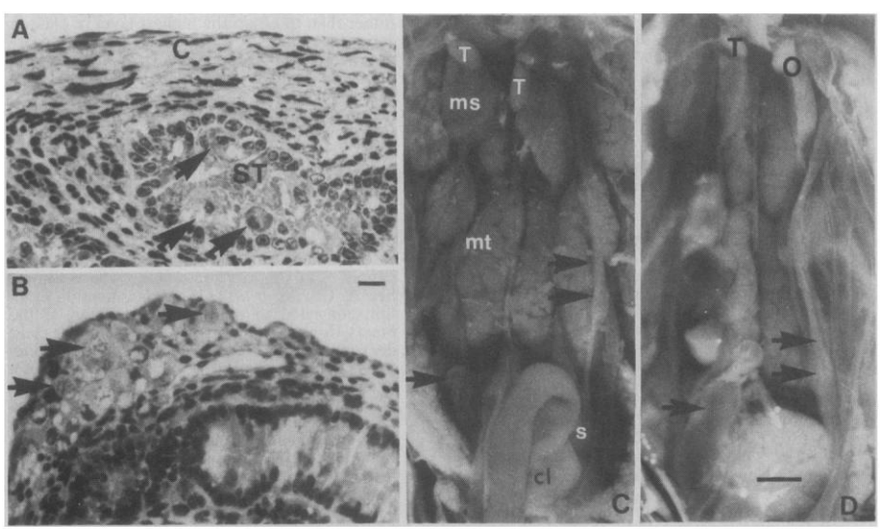

Fig. 1. (A) Section of normal testis of California gull at hatching. Seminiferous tubule (*ST*) contains PGC (arrows). Cortex (*C*) is squamous epithelium (×400). (B) California gull testis from egg injected with *o,p'*-DDT (2 ppm), showing cortical localization of PGC arrested in meiotic prophase (arrows) (×400; scale bar, 10 μm). (C) Reproductive organs of partially eviscerated male western gull hatchling injected during incubation with *o,p'*-DDT (5 ppm). Testes (*T*) are of normal shape. Feminization is indicated by the presence of left oviduct (double arrows), shell gland (*s*), and short right oviduct (single arrow). Other structures: mesonephros (*ms*), metanephros (*mt*), and cloaca (*cl*) (×3.5). (D) Reproductive organs of male western gull hatchling from egg injected with *p,p'*-DDT (10 ppm) and *p,p'*-DDE (40 ppm). Left gonad is enlarged and flattened into an ovotestis (*O*); left oviduct (double arrows) is present; right oviduct (single arrow) is prominent and edematous (×3.5; scale bar, 2 mm).

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of feminization by pollutants varied with compound and dose. The most sensitive indicator of feminization was localization of primordial germ cells (PGC) in a thickened ovary-like cortex in the left testis. The normal testis has a thin fibrous capsule surrounding the seminiferous tubules and interstitial cells (Fig. 1A). Estrogenic treatment results in a thickened cortex with clusters of PGC scattered throughout the cortex or localized into a ridge along the ventral border (*16, 17*). Higher doses result in intersexuality characterized by marked reduction in the number of seminiferous tubules and development of ovary-like cortical and medullary tissue. Primordial germ cells become clustered, but the cortex is less organized than in genetic females and the seminiferous tubules identify the intersexual gonads as those of genetic males.

Identification of PGC at hatching can be made with certainty. Germ cells in seminiferous tubules are large and have nuclei in interphase. Ovarian PGC, however, enter meiotic prophase during embryogenesis and are identifiable by their condensed chromatin. Primordial germ cells in the cortex of feminized testes also enter meiosis, providing a positive feminization marker. We adjudged testes as feminized only when PGC arrested in prophase were present in the cortex. The thickness of the cortex was somewhat variable in the two gull species and was not a reliable index of feminization.

The lowest feminizing doses resulted only in a thickened cortex and cortical localization of PGC (Fig. 1B). Higher doses resulted in testicular changes accompanied by the development of a left oviduct and shell gland, development of a right oviduct, reduced size of the right testis, and flattening of the left testis so that it approached the gross morphology of an ovary (Fig. 1, C and D).

The most estrogenic DDT isomer was *o,p'*-DDT. Seven of eight male embryos developed clusters of PGC in the cortex of the left testis at doses as low as 2 ppm. Doses of 5 ppm or higher caused development of both left and right oviducts. Metabolite *p,p'*-DDE caused localization of PGC in cortical tissue in half of the surviving male embryos at 20 or 100 ppm. The mixture of *p,p'*-DDE and *p,p'*-DDT (4:1) caused development of intersexual testes and oviducts at 50 ppm.

Methoxychlor, a less persistent organochlorine pesticide with estrogenic effects in mammals and birds (*18*), and HPTE [2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane], a highly estrogenic metabolite (*19*), also caused feminization. Cortical localization of PGC was induced at 2 ppm, and four of five male embryos surviving doses of 50 and 100 ppm developed left and right oviducts.

Estrogenic treatment of female embryos also results in developmental changes within the reproductive tract (*16, 18*). Development of the right oviduct in female hatchlings is abnormal and was induced by injections of methoxychlor, *o,p'*-DDT, and estradiol (Table 1). Three of seven female embryos injected with a mixture of *p,p'*-DDE and *p,p'*-DDT developed right oviducts.

The long-term effects of abnormalities induced in female birds by estrogen treatment have not been extensively studied. Effects on chickens were reported by Greenwood and Blyth (*17*), who found that hens raised from estrone-injected eggs had abnormal oviducts and laid eggs with thin or soft shells. Whether the developmental effects of organochlorine pollutants could be responsible for some of the observed eggshell thinning in wild birds is unknown.

The DDT contamination of southern California was primarily the result of industrial discharge into the sewer system. The major pollutant was commercial DDT containing 22 to 25 percent of the most estrogenic isomer, *o,p'*-DDT. Less persistent metabolites (*o,p'*-DDD, *p,p'*-DDD, and *o,p'*-DDE) also accumulated in fish and seabird eggs. The *o,p'* metabolites in particular averaged 23 percent of the DDT and DDD residues in more than 300 myctophid fish samples and were detected in brown pelican eggs at 0.5 ppm in 1969 (*2, 20*). The high level of nonpersistent compounds was somewhat unusual, as total DDT pollution from agricultural use normally consisted of an accumulation of *p,p'*-DDE.

The total DDT levels that caused feminization in this study were of the same magnitude as those found in seabird eggs in southern California in the late 1960's and early 1970's. The average total DDT content of brown pelican eggs from Anacapa Island in 1969 was 79 ppm (range, 40 to 141 ppm) (*20*). Double-crested cormorant eggs averaged 32 ppm (*6, 7*). Residues in gull eggs were not measured until after termination of DDT dumping into the Los Angeles sewer system. In 1973, 3 years after dumping had ceased, gull eggs contained 6.3 to 30 ppm total DDT—sufficient amounts to cause abnormal development.

Feminization of male avian embryos markedly affects the reproductive behavior of the birds when they mature. Adkins (*21*) and Whitsett *et al.* (*22*) demonstrated that injection of estrogen (0.2 to 5 ppm) into chicken and Japanese quail eggs during incubation results in permanent suppression of crowing, strutting, and copulatory behavior. Suppression of breeding behavior in male gulls could reduce migration to the breeding colony and result in the markedly skewed sex ratio of adult birds observed on Santa Barbara Island. It is unlikely that any of

Table 1. Compounds injected before incubation and morphology of surviving gull embryos at hatching.

Compound	Dose* (ppm)	Embryo morphology				Eggs injected§
		Males		Females		
		Normal	Feminized†	Normal	Abnormal‡	
Control		10	0	20	1	46
o,p'-DDT						
Low	2, 5	1	5‖	2	2	24
High	20, 50, 100	0	2¶	0	5**	26
p,p'-DDT						
Low	2	2	0	2	0	4
High	20, 100	2	0	3	0	11
p,p'-DDE						
Low	2	1	0	1	0	4
High	20, 100	3	3¶	2	0	10
p,p'-DDE and *p,p'*-DDT (4:1)						
Low	5	1	1	2	1	18
High	50	0	2¶	2	2	18
Methoxychlor						
Low	2, 5	0	3‖	1	1	22
High	20, 50, 100	0	5**	0	6**	27
Estradiol						
Low	0.5, 2	0	5**	1	3‖	27
High	2, 5	0	5**	1	0	27

*Dosage data are combined into high and low groups because of the small number of artificially incubated embryos. †Males with PGC in cortex of left testis and males with oviducts. ‡Females with both left and right oviducts. §Does not include eggs damaged during injection. Embryo mortality (mean, 59 percent) can be determined by subtracting the number of surviving chicks. ‖Significantly different from corresponding control value at $P < .01$ (Fisher's exact test). ¶$P < .05$. **$P < .001$.

the excess females might be sex-reversed males, as complete reversal (with achievement of egg-laying capability) has not been observed in experiments with any bird species.

Reproductive failure due to developmental suppression of male breeding behavior could remain unrecognized for years, especially in resident populations, because although birds are conspicuous throughout the year, nesting is usually secretive. Only in breeding colonies might the abnormality become evident and then perhaps only indirectly. The highly skewed sex ratio of gulls on Santa Barbara Island, for example, was discovered only because of questions raised by the unusual female-female pairing.

Abnormal development induced by DDT in birds could be more persistent than the pollutant itself. Breeding failure of unexplained origin in birds other than gulls may be the result of DDT contamination of eggs.

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Structural Correlates of Seizure Behavior in the Mongolian Gerbil

Abstract. *Hippocampi of seizure-sensitive and seizure-resistant Mongolian gerbils were examined in search of structural correlates of seizure behavior. In animals with well-established seizure histories, differences were found in both presynaptic and postsynaptic structures. Seizing animals had less dense dendritic spines, a greater proportion of mossy tuft area devoted to presynaptic vesicles, and a smaller proportion devoted to spines. The possible relationship of these findings to epilepsy is discussed.*

Attempts to characterize an anatomical substrate for epilepsy have yet to yield definitive results. Conclusions from qualitative studies on monkeys after application of toxic substances to the cortex and on humans undergoing temporal lobectomy for chronic seizure states (*1*) have been limited by the uncontrolled nature of tissue damage, while qualitative data from genetic models [photosensitive baboon (*2*); sound-sensitive mouse (*3*)] have not been reported. We have investigated seizure substrates in the Mongolian gerbil (*Meriones unguiculatus*), an animal that exhibits spontaneous seizures (*4*). Major advantages of this animal as a model for epilepsy—including the relative ease of eliciting

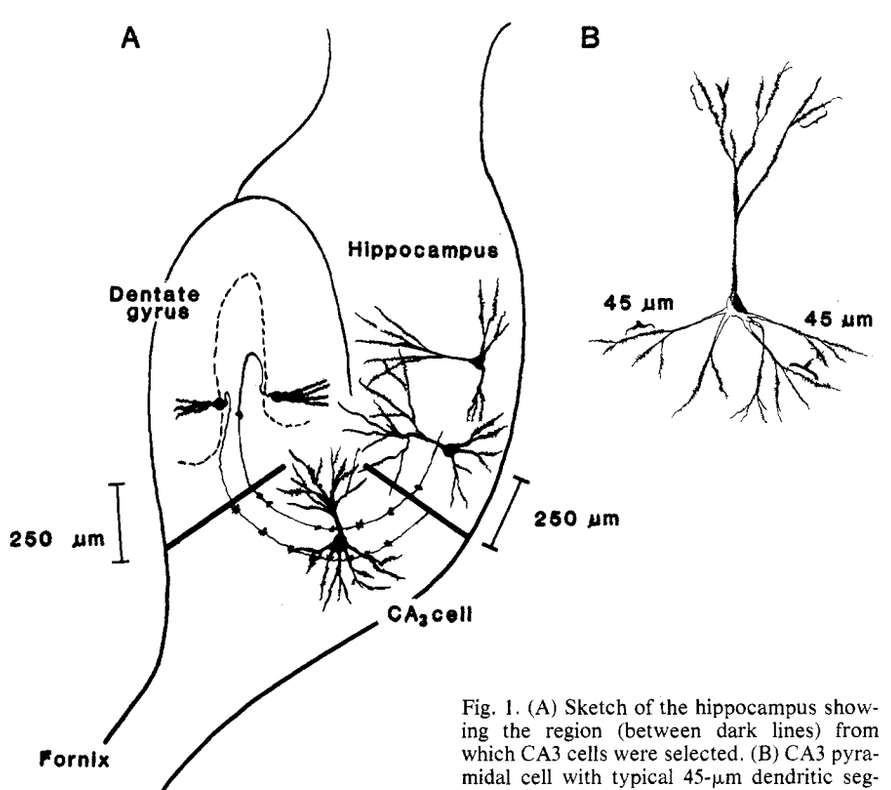

Fig. 1. (A) Sketch of the hippocampus showing the region (between dark lines) from which CA3 cells were selected. (B) CA3 pyramidal cell with typical 45-μm dendritic segments used for spine counts.

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