which probably are chemoreceptors (*4*), while some photographs of *Acetes* following trails show antennules raised above the trail which impinges on the mouth parts and proximal sections of the pereiopods.

Acetes manifests its fine-tracking response only in particular laboratory situations. In smaller aquariums than those used, the animals are too agitated to respond, and in cylindrical containers they aggregate against the side. Properly selected laboratory conditions probably will show that the ability to track scent trails is widespread throughout planktonic taxa. Copepods (*Calanopia elliptica*) and a reef lagoon mysid (as yet unidentified) both follow scent trails in our aquariums.

A field observation made during research in the Florida Current (*6*) was partly responsible for these experiments and indicates that scent trails occur naturally in the open sea. Diving to a depth of 15 m, we collected samples of a "remarkable amorphous material" covered with copepods. Upon surfacing we found that a boat tender had become seasick and regurgitated a partially digested meal, part of which was deposited in the water. The falling particles probably had produced discrete scent trails that quickly attracted surprisingly large numbers of copepods.

The genus *Acetes* lives in coastal waters (*9*), which often provide little or no visibility and where chemosensory cues should be extremely important. In the perpetual dark of the deep sea, scent trail-following may be an even more important behavioral response. Perhaps the deep sea, with its low turbulence, is laced with a complex array of attractive or repellant chemical trails. If so, scents may prove to be even more important for aquatic animals than they are for terrestrial organisms.

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Paradoxical Effects of Amphetamine on Preweanling and Postweanling Rats

Abstract. *In adult rats amphetamine acts as a strong behavioral stimulant leading to a marked increase in random, nondirected locomotor activity. In contrast we report that amphetamine administered to preweanling rats in the presence of an anesthetized adult rat produces no visible increase in motor activity. Instead, it appears to enhance the normal tendency of neonatal rats to approach and maintain contact with conspecifics. In postweanling rats amphetamine disrupts the tendency to aggregate and produces an increase in behavioral activity comparable to that seen in adult rats. These findings may constitute the basis for an animal model of minimal brain dysfunction hyperkinesis.*

Minimal brain dysfunction (MBD) is one of the most common clinical syndromes of childhood. It is characterized by high levels of motor activity, short attention span, nonresponsiveness to social influences, and lack of impulse control (*1*). One of the most unusual aspects of MBD is the calming effect that stimulants, especially amphetamine and methylphenidate, have on the hyperkinetic child. The mechanisms of action for this paradoxical calming effect are unknown. Numerous models of both MBD and the paradoxical calming effect of amphetamines have been put forth, but none have received general acceptance (*2*).

A widespread view, however, is that MBD represents a lag in some aspect of neurological development rather than a permanent impairment (*3*). The behavioral profile of MBD is reminiscent of the preschool child; moreover, the syndrome typically disappears as the child enters late adolescence. Unfortunately, it is not known whether amphetamine has a calming effect on preschool children during their period of normal hyperkinesis, and such research is now precluded for ethical reasons. Most altricial mammals, however, pass through comparable periods of hyperactivity during ontogenesis (*4*); the developing normal mammal may therefore serve as an animal model of hyperkinesis.

In this report we describe the effects of amphetamine on normal rats during various stages of development, when tested either in isolation or in their natural environment. In the nest, preweanling rats spend most of their time in close physical contact with siblings and the dam, and, given the opportunity, will orient toward, approach, and remain in contact with those conspecifics (*5*). In preweanling rats (15 days of age) the response to amphetamine is an enhancement of these approach and contact behaviors. Later in development (30 days of age) amphetamine disrupts the normal tendency of rats to aggregate and produces its characteristic increase in random, nondirected motor activity. These data suggest that stimulants serve to direct or "canalize" behavior during certain stages of normal development, thereby producing its apparent calming effect.

The basic experimental procedure was to compare the amount of locomotor activity elicited by various doses of amphetamine administered to 15- and 30-day-old rats tested either alone or in the presence of an adult, anesthetized male rat. Time spent in contact with the anesthetized male was also recorded. Previous research had shown that this preparation elicits vigorous approach and contact behavior in the preweanling rat (*6*).

The subjects were 160 albino rats of Sprague-Dawley descent, bred and raised in the Princeton University colony. Rats were randomly assigned to 20 equal groups in a factorial combination of age (15 or 30 days), dose (0, 0.25, 0.5, 1.0, or 2.0 mg of *d*-amphetamine sulfate per kilogram of body weight), and test condition (tested in isolation or in the presence of an anesthetized adult). All doses were calculated as the sulfate salt dissolved in isotonic saline. Control animals were injected with the saline vehicle only.

All observations were made in four polypropylene rat cages (25 by 46 cm), the floors of which were covered with dried beet pulp. Neither food nor water

was present during the session. For half the groups an anesthetized (urethane, 1.2 g/kg) adult male was placed across one end of the test box.

The behavior of the animal was recorded by means of a television camera mounted directly above the four cages and attached to a time-lapse video tape recorder (Concord, model 624; record, 1.5 fields per second; playback, 60 fields per second). Subjects were placed in the box for a 60-minute adaptation period and then given an intraperitoneal injection of isotonic saline or the appropriate dose of *d*-amphetamine.

Following the injection, activity was monitored for ten 20-minute periods. The floor of each cage was divided into two equal parts by a line across the shorter axis of the cage on the television monitor, and activity was recorded in the appropriate groups for the same ten 20-minute periods.

The effects of amphetamine on locomotor activity in 15- and 30-day-old rats tested in isolation are shown in the left-hand panels of Fig. 1. Fifteen-day-old rats become much more active in response to amphetamine and respond to lower doses than do 30-day-old rats. These results confirm and extend earlier findings (*7*) on the effects of amphetamine administered to young rats on locomotor activity in stabilimeter cages. The duration of the stimulant effects of amphetamine is also longer in the 15-day-old, possibly because of slower metabolism of the drug (*8*).

From data in the right-hand panels of Fig. 1 it is obvious that the anesthetized adult rat completely inhibits the marked increase in locomotor activity produced by amphetamine in the isolated 15-day-old but does not decrease the activity of 30-day-old rats.

Analysis of variance confirms these observations. Age, test condition, dose, and their interactions all contribute significantly to the between subjects variance (all P's $< .01$). Furthermore simple effects analyses show that dose was significant within both the 15- and 30-day groups ($P < .01$ and $P < .05$, respectively). Test condition and the interaction of dose and test condition were significant in the 15-day-old group ($P < .01$ for both) but not the 30-day-old group ($P > .10$).

An additional component of the drug response is presented in Fig. 2, which shows the percentage of time spent in contact with the anesthetized animal. At all doses of *d*-amphetamine 15-day-old rats stay in almost constant contact with the anesthetized adult, while 30-day-old animals show a dose-related decrease in contact time. The latter result replicates earlier findings (*9*) which show that amphetamine disrupts conspecific aggregation in adult rats.

These results suggest that, in the neonate, increases in behavioral arousal are canalized into biologically meaningful response patterns when appropriate stimuli are available, but that when no such stimuli are present a state of supranormal behavioral excitement is evoked. Later in development the same stimulant condition produces strong increases in nondirected behavioral excitement and disrupts the normal tendency of adult rats to aggregate.

To further illustrate this principle, we administered amphetamine to 15- and 30-day-old animals in the presence of an anesthetized adult rat that was moved approximately 30 cm every 2 minutes. The apparatus consisted of a circular open field 61 cm in diameter containing a motor-driven arm that pulled the anesthetized adult around the perimeter of the field on a Plexiglas plate. The motor (1 rev/min) was activated every 2 minutes for 15 seconds, moving the anesthetized animal 90° around the perimeter. The anesthetized adult was covered with a piece of Plexiglas, leaving only the lateral flanks exposed, to prevent the pups from riding directly on it.

Under these circumstances 15-day-old rats injected with saline spent relatively little time in contact with the periodically moving animal. Instead, after a few abortive efforts to stay next to it, they appeared to fall asleep or become quiescent. Also, they were not as active in the presence of the moving anesthetized animal as they were when placed alone in the apparatus. When these animals were given amphetamine, however, a very different pattern of behavior emerged. Each time the anesthetized animal was pulled a quarter turn, the amphetamine-treated animal became alert, engaged in a burst of activity until it regained contact, and then either became quiescent or indulged in what appeared to be vigorous rooting and/or investigative behavior.

The second experiment consisted of 80 Sprague-Dawley rats as previously described. They were assigned, eight per group, to doses of 0.00, 0.25, 0.5, 1.0, and 2.0 mg of *d*-amphetamine per kilogram and were 15 or 30 days of age. An adaptation period of 1 hour was followed by an intraperitoneal injection of the appropriate dose of *d*-amphetamine sulfate

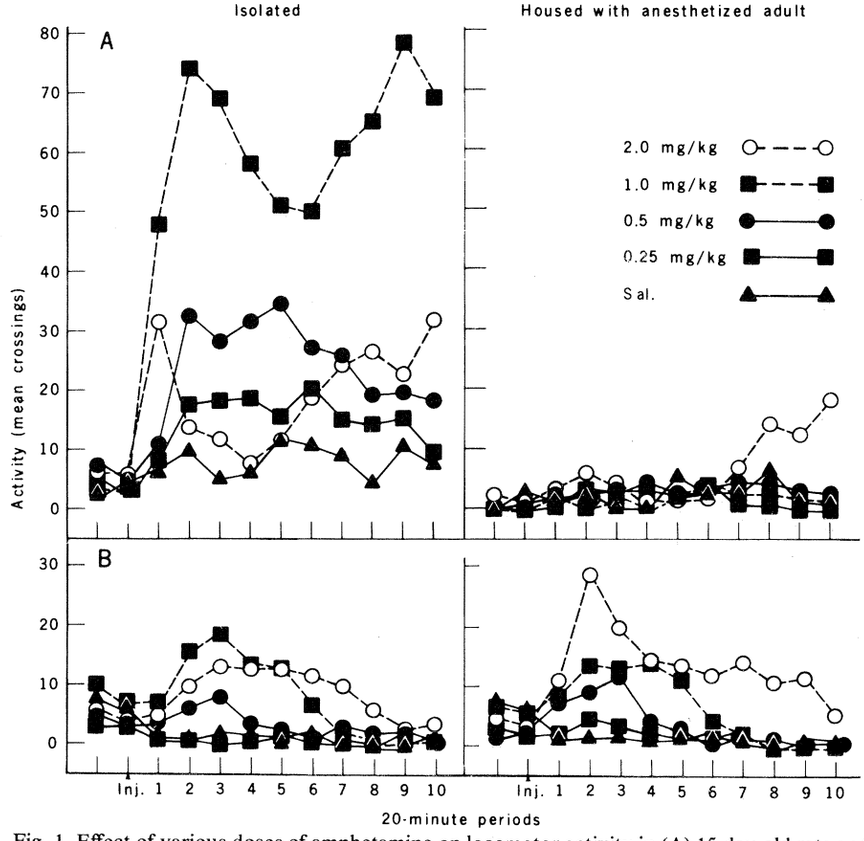

Fig. 1. Effect of various doses of amphetamine on locomotor activity in (A) 15-day-old rats and (B) 30-day-old rats tested alone or in the presence of an anesthetized adult male rat; *Sal.*, saline; *Inj.*, injection.

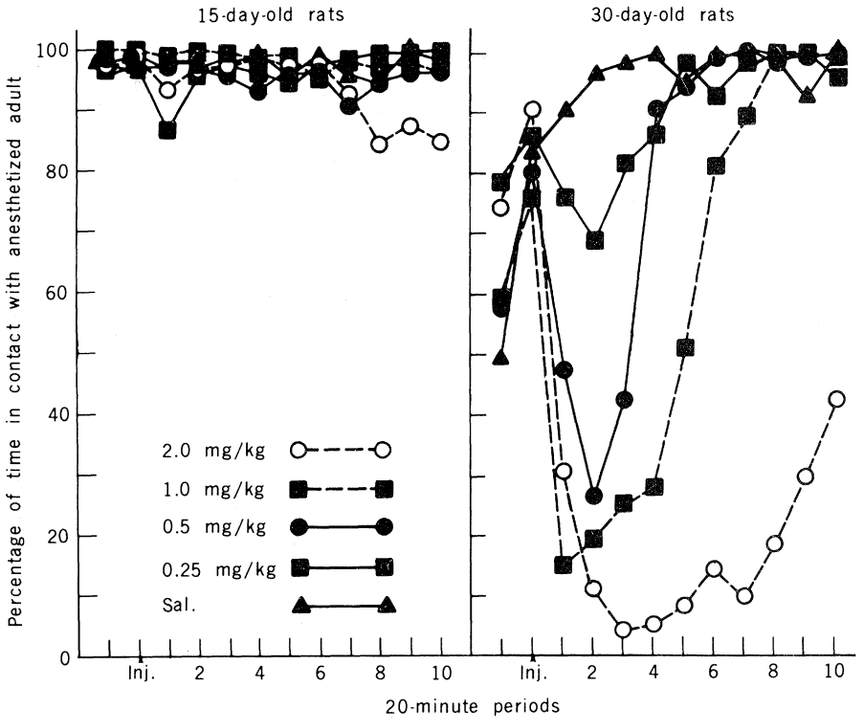

Fig. 2. Effect of amphetamine administered to 15- and 30-day-old rats on time spent in contact with an anesthetized adult male rat; *Sal.*, saline; *Inj.*, injection.

or saline, and behavior was recorded on time-lapse video tape. Time spent in contact with the anesthetized adult was recorded for ten successive 20-minute periods.

The results of this experiment are presented in Fig. 3, which shows the percentage of time spent in contact with the periodically moving stimulus animal. Fifteen-day-old animals show a dose-related increase in contact behavior. The 30-day-old animals, however, showed little or no tendency to increase contact at any dose. Analysis of variance on these data shows that dose, age, and the interaction of dose and age were highly significant (all P's $< .01$).

This pattern of results further supports the view that amphetamine directs or canalizes arousal toward ethologically relevant stimuli in the neonate. Amphetamine-treated animals orient and move directly toward the stimulus animal after it has been moved. Thus the presence of the adult animal does not merely inhibit arousal in amphetamine-treated neonates; instead, it appears to potentiate the normal approach and contact responses characteristic of that developmental stage. In the postweanling rat canalization of arousal toward conspecifics does not occur (see Fig. 3).

The transition from conspecific-directed arousal to nondirected arousal during development is probably dependent on the continuing maturation and differentiation of the central nervous system. The approach and contact responses potentiated by amphetamine are probably analogs of other, more tangible reflexive behaviors of infancy. During the normal course of development reflexes such as rooting, suckling, crossed extensor, grasping, and Babinski are actively inhibited by the forebrain as it becomes functionally mature. In man and other mammals, damage to the cortex and other forebrain regions result in reappearance of the reflexes of infancy by removing continuously acting sources of inhibition (*10*). In our view the approach and contact elicited by amphetamine in preweanling rats consists of a mélange of reflex-like, species-typical behaviors that are actively antagonized by maturation of the forebrain during the normal course of development. Prior to this inhibition, amphetamine augments those infantile species-specific behaviors.

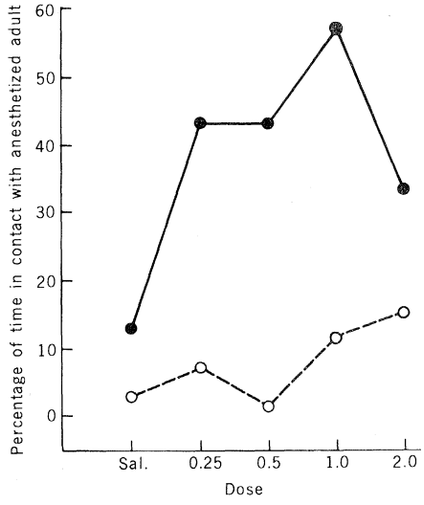

Fig. 3. Effect of amphetamine administered to 15- and 30-day-old rats on time spent in contact with the periodically rotated anesthetized adult rat. Doses of *d*-amphetamine are given in milligrams per kilogram of body weight. Closed circles, 15-day-old rats; open circles, 30-day-old rats; *Sal.*, saline.

These results may have significant implications for the "paradoxical" effects of amphetamine in hyperkinetic children. Our research demonstrates that amphetamine enhances some naturally occurring neonatal behaviors that normally disappear during the development. Thus the response to amphetamine is qualitatively different in the infant rat than in the adult. In man a similar pattern of development may occur. In the normal child amphetamine may activate a set of infantile behaviors, including directing attention toward adults, which are qualitatively distinct from those energized in the adult. By directing behavior, amphetamine, when administered under the appropriate circumstances, may produce a "calming" effect in the normal preschool child, but later in development produces the heightened excitation characteristic of the drug's effect on adults.

In minimal brain dysfunction this transition from the infantile to adult pattern of responding to amphetamine may be slowed, along with a maturational lag in some aspects of emotional development. To the extent that amphetamine produces apparent calming of the hyperkinetic child, it may reflect the elicitation or potentiation of specific behaviors characteristic of an immature maturational stage in the normal child.

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Superhelix Densities of Circular DNA's: A Generalized Equation for Their Determination by the Buoyant Method

Abstract. *Equations for the measurement of the supertwisting of circular DNA's from banding positions in buoyant density gradients containing intercalating dyes have required the use of SV40 DNA isolated from virions as a reference DNA. These equations are modified to allow the use of any closed circular DNA of known superhelix density as a reference DNA.*

Covalently closed duplex circular DNA (duplex circular DNA containing no single strand scissions) was first characterized in 1963 by Weil and Vinograd (*1*). Since that time a large number of DNA's with similar properties have been found, including the *Escherichia coli* chromosome, various prokaryotic and eukaryotic viral and plasmid DNA's, and organelle DNA's. Alteration of the duplex winding of closed circular DNA's causes tertiary conformation changes (supertwists or superhelical turns) as a result of the topological constraint present in such DNA's. Recently, both Griffith and Germond *et al.* have shown a relation between chromatin structure and the number of superhelical turns present in closed circular DNA (*2*). Others have suggested that the degree of supertwisting may be important in replication (*3*), transcription (*4*), and recombination (*5*) of DNA. The topological properties giving rise to superhelical turns are not limited to circular DNA's but are also relevant for linear DNA's lacking single-strand discontinuities and whose ends are fixed so that they cannot rotate about each other.

Several methods for the determination of the superhelix density (σ) of closed circular DNA's have been developed (*6*). One of these, the measurement of separations between bands of closed and open circular DNA's in buoyant gradients containing high concentrations of the intercalating dyes ethidium bromide or propidium diiodide (*7, 8*) has been widely used since it is possible to determine σ by this technique in a single buoyant density separation in a preparative ultracentrifuge. This procedure is also ideal for determinations in which radioactively labeled DNA is used (*9*).

A little known limitation of the buoyant separation method (*7, 8*) is that only SV40 DNA or a DNA with an equal superhelix density may be used as a reference DNA. The σ_0 for SV40 (the subscript zero refers to standard measurement conditions of 2.8*M* CsCl, 20°C, and neutral *p*H) was used in the derivation of the equations used in this method (*7*, appendix, equations I-1, I-8, and I-9) and SV40 DNA was the reference DNA for the experimental verification of this procedure (*8*).

The published equations can be easily generalized for use with any closed circular DNA of known σ_0 as a reference DNA. The previously published equation (*8*) is

$$\Delta\sigma_0^{\text{unk-SV40}} = \sigma_0^{\text{unk}} - \sigma_0 = a\left(\frac{\Delta_r^{\text{unk}}}{\Delta_r^{\text{SV40}}} - 1\right) \quad (1)$$

where $\Delta\sigma_0^{\text{unk-SV40}}$ is the difference in superhelix density between the unknown DNA (σ_0^{unk}) and SV40 (σ_0^{SV40}), Δ_r^{unk} and Δ_r^{SV40} are the distances between bands of closed and open circular DNA's for unknown and SV40 DNA's, and a is a constant. [Correction factors for differences in base composition between unknown and reference DNA's and for differences in banding positions in individual tubes are omitted here, but they are available in (*6–8*)]. If σ_0^{ref} and Δ_r^{ref} are substituted in Eq. 1 for σ_0^{unk} and Δ_r^{unk} to generate a second equation, Δ_r^{SV40} may be eliminated to give the general form of the equation for use with any reference DNA

$$\Delta\sigma_0^{\text{unk-ref}} = \sigma_0^{\text{unk}} - \sigma_0^{\text{ref}} = (b + \sigma_0^{\text{ref}})\left(\frac{\Delta_r^{\text{unk}}}{\Delta_r^{\text{ref}}} - 1\right) \quad (2)$$

where $\Delta\sigma_0^{\text{unk-ref}}$ refers to the difference in superhelix density between the unknown DNA and the reference DNA. The factor a in Eq. 1 is replaced by the sum of two independent factors, $b + \sigma_0^{\text{ref}}$, where b is dependent on the intercalating agent and σ_0^{ref} is the superhelix density of the reference DNA.

The value of a in the original equation was computed on the assumption that the helix unwinding angle per molecule of intercalated ethidium bromide was 12° (*10*). More recent data (*11*) indicate that 26° is a better value for this unwinding angle, and thus values of b for separations measured in CsCl gradients containing ethidium bromide or propidium diiodide are, respectively, 0.33 and 0.29. The corrected value of σ_0 for SV40 is −0.084. The PM2 DNA (corrected value of $\sigma_0 = 0.11$) is a good alternative reference DNA as it is easily prepared and its σ_0 has been well characterized (*8, 12*). Intracellular forms of SV40 or PM2 DNA's should not be used as they are more heterogeneous in σ_0 compared with DNA isolated from virions (*9, 13*). Specific details regarding superhelix determinations by this method have been described (*6, 8*). In calculating the number of potential superhelical turns in any DNA, differences between in vivo and measurement environmental conditions (such as salt and temperature) must also be considered (*14*).

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