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Genetic and Ontogenetic Variation in Response to Scopolamine and D-amphetamine in Three Strains of Mice

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I hereby declare that I am the sole author of this thesis. I authorize Wilfrid Laurier University to lend it to other institutions or individuals for the purpose of scholarly research.

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Abstract

Highly inbred mice of three strains (A/J, DBA/2J and C57BL/6J) were tested in an activity task at 14, 21, or 28 days of age. Ten minutes prior to testing, mice received treatment of either saline, scopolamine (0.5 or 1.0 mg/kg) or d-amphetamine (0.5, 1.0 or 5.0 mg/kg). At 14 days of age DBA mice exhibited higher levels of activity than either A or C57 mice. However, the increase in activity in C57 was such that at 21 and 28 days no difference was observed relative to DBA mice, and activity was higher than that of A mice. With respect to the drug treatments, d-amphetamine (5.0 mg/kg) increased activity in all strains at 14 days and 28 days of age. At 21 days, only C57 exhibited lack of a significant increase in activity. Clearly, the system responsible for responsivity to d-amphetamine (dopamine and norepinephrine) is mature at 14 days of age. In contrast to d-amphetamine, a response to scopolamine was evident in DBA at 21 days of age, and in C57 and A at 28 days of age. These data were taken to support a caudal-rostral gradient of brain development, with the inhibitory cholinergic system developing more slowly than the excitatory catecholamine system. Moreover, there also appears to be strain-specific differences in neuronal development. The differential development of the cholinergic system, together with potential differences in the ontogeny of the catecholamine system, were suggested as being responsible for age dependent variations in activity, as well as differential responsivity to pharmacological agents which increase catecholamine activity.

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INTRODUCTION

There is little debate regarding the degree of maturity of the central nervous system at birth. While considerable organization and proliferation of neurons occurs prenatally, very rapid growth and development of synaptic junctions occur in cortical and subcortical areas in the rat and mouse brain during the third and fourth postnatal weeks (Aghajanian & Bloom, 1967). In addition, rapid dendritic growth in the mouse brain has been recorded between 8 and 15 days postnatally (Himwich, 1970). Similarly, the maturation of neurotransmitter systems probably approximates the sequence of synaptic development, since the storage and release of these endogenous chemicals are directly related to presynaptic functioning (Axelrod, 1970). Indeed, Keller, Bartholini, and Pletscher (1973), Lander and Bloom (1974), Loizou (1971), and Loizou and Salt (1970) have identified increased levels of brain monoamines in close temporal proximity to the proliferation of axon terminals of monoamine containing neurons. Although most rapid ontogenesis of the central nervous system occurs during the third and fourth postnatal weeks, further development continues up to at least one year of age (Pscheidt & Himwich, 1966). Of course, the precise nature of the growth is species dependent, with precocial organisms such as the guinea pig (Bennett & Giarman, 1965) and chick (Pscheidt & Himwich, 1966) showing greater prenatal development than that seen in altricial organisms such as the rat (Eiduson, 1971).

Unlike the development of the peripheral nervous system, which follows a proximo-distal and cephalo-caudal pattern, numerous studies have indicated agreement with the Jacksonian model of brain organization (Jackson, 1931).

That is, there exists a caudal-rostral gradient of brain development with the higher (rostral) regions developing more slowly than the lower (caudal) regions (Eiduson, 1971; Kim, Choi, Kim, Chang, Park, & Kang, 1970; Moorcroft, 1971). Moreover, it is well known that the rostral structures, such as the limbic forebrain (including septum, hippocampus, and amygdala) are largely modulated by acetylcholine which inhibits ongoing activity, while caudal areas, such as hypothalamus, are exceptionally rich in catecholamines (dopamine and norepinephrine) which tend to have excitatory effects. Areas between these structures, such as the caudate-putamen, contain large amounts of both acetylcholine and dopamine (Cooper, Bloom, & Roth, 1970). In effect, the suggestion has been that the inhibitory cholinergic system may interact, either in an additive or synergistic manner, with the excitatory catecholaminergic system (Campbell & Mabry, 1973; Carlton, 1963, 1969; Feigley, 1974; Moorcroft, Lytle, & Campbell, 1971).

With respect to the development of the neurotransmitter systems, several researchers have documented the slower development of cholinergic pathways than of pathways involving catecholamines, with particular reference to norepinephrine. These observations have come primarily from experiments evaluating enzymatic activity associated with various transmitters in both rats (Coyle & Axelrod, 1972a, 1972b; Loizou, 1971; McGeer, Fibiger, & Wickson, 1971; Mellgren, 1973; Porcher & Heller, 1972) and mice (Baker, Hoff, & Smith, 1973). For example, it has been observed that levels of monoamine oxidase (MAO), the enzyme which degrades monoamines (dopamine, norepinephrine, and serotonin) intracellularly, dopamine-β-hydroxylase,

the enzyme involved in the conversion of dopamine into norepinephrine (Coyle & Axelrod, 1972a), as well as tyrosine hydroxylase, the rate limiting step in the synthesis of catecholamines (Coyle & Axelrod, 1972b; Porcher & Heller, 1972), increase during postnatal development (Baker & Hoff, 1972; Coyle & Axelrod, 1971; Haber & Kamano, 1966; Iverson, de Champlain, Glowinski, & Axelrod, 1967; Loizou & Salt, 1970; Robinson, 1968). Of course, the presence and effectiveness of the various neurotransmitters is related to the functional maturity of these enzymes. The presence of these enzymes appears in detectable quantities in the rodent brain at 15 days of gestation. In contrast, enzymes associated with acetylcholine synthesis or degradation (choline acetyltransferase and acetylcholinesterase, respectively) reach adult levels between 15 and 20 days of age postnatally (Hattori & McGeer, 1971), clearly revealing slower development than enzymes associated with the amines (McGeer et al, 1971; see also Ladinsky, Consolo, Peri, & Garattini, 1972). While the data regarding enzymatic ontogeny have been useful in elucidating neuronal and neurochemical development, a number of studies have been reported which demonstrate that responsivity to pharmacological treatments may similarly lend themselves to ontogenic analysis of endogenous chemicals, as well as their role in the maturation of behaviour (Campbell, Lytle, & Fibiger, 1969; Fibiger, Lytle, & Campbell, 1970; McGeer et al, 1971; Thornburg & Moore, 1973). This particular technique has three advantages over the biochemical assays described, the first simply being that the research can be carried out more quickly and efficiently. Secondly, pharmacological effects of various treatments can be evaluated concomitantly, and finally,

interactions among various systems can be detected. In the following sections, investigations of this nature, together with their rationale, are documented.

Behavioural Effects of Acetylcholine and Catecholamines

It has been reported by a number of investigators that modifications of neurotransmitter levels by pharmacological manipulation have profound effects on a number of behavioural measures. For example, agents which effectively decrease levels of acetylcholine, such as scopolamine and atropine, tend to enhance active avoidance (Anisman, 1973; Anisman, 1974a; Anisman & Kokkinidis, 1974; Bignami & Rosic, 1971; Bignami, Amorico, Frontali, & Rosic, 1971; Carlton, 1962; Carlton & Markiewicz, 1971; Safer & Allen, 1970) increase levels of general activity (Anisman, 1974b; Anisman, Wahlsten, & Kokkinidis, 1974; Payne & Anderson, 1967; Schwartzbaum, Ide-Johanson, & Belgrade, 1974), decrease passive avoidance (Feigley, 1974), and eliminate or reduce habituation tendencies (Anisman, 1974b; Anisman & Kokkinidis, 1974; Carlton, 1961; Carlton & Vogel, 1965). The converse is true of agents which increase levels of acetylcholine e.g. physostigmine or pilocarpine (see, for example, Anisman, 1974; Bignami & Rosic, 1970; Bignami, Rosic, Mickalek, Milosevic, & Gatti, 1974, in press). Manipulations of dopamine and norepinephrine have also been observed to modify behaviour. Decreasing levels of these amines tends to disrupt avoidance and reduce locomotor activity (Cooper, Grant, & Breese, 1973; Costa & Groppetti, 1970; Ellison & Bresler, 1973; Fibiger, Fibiger, & Zis, 1973; Herman, Trzeciak, Chrusciel, Kmieciak-Kolada, Drybanski, & Sokola, 1971; Neill, Boggan, & Grossman, 1974), while increases in catecholamine activity with

drugs such as amphetamine (Goodman & Gillman, 1965) augment avoidance performance and increase general activity (Anisman, 1974a; Axelrod, 1974; Bindra & Baran, 1959; Bindra & Mendelson, 1963; Cole, 1967; Grossman & Scalfani, 1971; Rech & Stolk, 1970; Van Rossum, 1970; see reviews in Costa & Garattini, 1971). Moreover, over the past few years, advances have been made in differentiating behavioural properties of dopamine and norepinephrine. For example, dopamine appears to be involved in producing stereotypic behaviours (Anden, Srombom, & Svenson, 1973; Fog, Randrup, & Pakkenberg, 1967, Molander & Randrup, 1974; Randrup & Munkvad 1967, 1968, 1970) and in modulating activity levels (Maj, Grabowska, & Mogilnicka, 1971; Thornburg & Moore, 1973; Ungerstedt, 1971, 1974). Norepinephrine is also involved in moderating activity, but to a lesser extent than that of dopamine (Dominic & Moore, 1971; Rolinski & Scheel-Kruger, 1973), and is also involved in changes in activity levels with the inception of stressors (Anisman & Cygan, 1974). These conclusions are based on numerous studies indicating that synthesis inhibition of dopamine and norepinephrine, via α-methyltyrosine, completely abolishes the excitatory effects of amphetamine. However, inhibition of norepinephrine, while leaving dopamine synthesis intact, by bis-(4-methyl-1-homopiperazinylthio-carbonyl) disulphide (FLA-63), the inhibitor of dopamine-β-hydroxylase, has lesser effects on increases in activity promoted by amphetamine (Carlsson, 1970). Some of the more recent positions have involved the suggestion that dopamine, in fact, modulates activity levels; however, this is dependent on the presence of adequate levels of norepinephrine (Carlsson, 1970).

In light of the polar effects of cholinergic and noradrenergic drugs, it has been suggested that a distinct balance exists between the catecholamine excitatory system (dopamine and norepinephrine) and the inhibitory cholinergic system (Carlton, 1963, 1968, 1969; McGeer et al, 1971). According to this hypothesis, there exists a cholinergic mechanism in the brain that antagonizes a dopaminergic and/or noradrenergic system, which is excitatory. The notion that these 2 systems act in a specific balance is strongly supported by studies involving drug interactions. For example, it has been demonstrated that low, normally non-effective doses of scopolamine can augment the action of amphetamine in increasing responses within an active avoidance situation (Carlton, 1961; Carlton & Didamo, 1961). Furthermore, it has been found that cholinomimetic drugs, such as pilocarpine, decrease amphetamine induced psychomotor excitation in rats (Carlton, 1961). Evidence has been gathered which would suggest that there is an interaction between peripheral epinephrine effects and the central cholinergic and noradrenergic systems. Specifically, injections of scopolamine are able to prevent reductions in motor activity and avoidance ordinarily elicited by epinephrine (Manto, 1967; Remington & Anisman, 1974).

Summarizing then, there are ample data indicating that the cholinergic system inhibits active behaviours while the catecholamine system activates these behaviours. Moreover, these systems act in a reciprocal antagonistic manner. Extension of these studies involving direct application of anticholinergics to limbic structures (Abeelen, Smits, & Raaijmakers, 1970; Abeelen, Gilissen, Hanssen & Lenders, 1971; Grossman, 1962, 1964; Grossman

& Scalfani, 1971), or dopaminergic and noradrenergic agents to the substantia nigra and hypothalamus, respectively (Fibiger et al, 1973; Grossman & Scalfani, 1971) have yielded results comparable to those witnessed with systemic injections. Such studies have thus implicated these structures as being involved in inhibitory or excitatory processes, and have clearly shown the drug actions to be primarily of a central rather than a peripheral nature.

Ontogeny of Behaviour: Pharmacological Manipulations

From a behavioural vantage, the available data have supported the Jacksonian model of brain organization, and have also implicated catecholamine and acetylcholine ontogeny as factors in modulating age-dependent changes in behaviour. For example, in precocial organisms such as the guinea pig (Campbell & Mabry, 1972; Himwich, 1970; Karki, Kuntzman, & Brodie, 1962) and chick (Campbell & Mabry, 1972; Masai, Kusunoki & Ishibashi, 1965), where the brain is fairly well developed at birth, little variation in activity levels is seen from 5 to 100 days of age. In contrast, among altricial organisms such as the rat (Moorcroft et al, 1971) or hamster (Campbell & Mabry, 1972), a sizeable increase in activity is observed between 10 and 15 days postpartum, followed by a substantial decline in activity between 15 and 25 days of age. It will be recalled that these ages are roughly comparable to the caudal-rostral pattern of brain development in which the inhibitory telencephalic centres mature later than the brain stem excitatory areas. Surgical manipulation of various brain structures have substantiated the role of limbic and hypothalamic structures in the ontogenic variations in locomotor behaviour.

That is, surgical removal of the hippocampus, which appears to be a major axis for acetylcholine activity (Kelsey & Grossman, 1969; Lewis, Shute, & Silver, 1967; Lynch, Mathews, Mosko, Parks & Cotman, 1972; Macadar, Roig, Monti & Buddeli, 1970; Mellgren & Srebro, 1973), does not appear to affect behaviour in the rat prior to 21 days of age, but produces marked increases in activity among adult animals (Moorcroft, 1971).

In terms of responsivity to drug treatments, it has been observed repeatedly that administration of amphetamine augments activity levels in rats as early as 10 days of age, whereas scopolamine, which blocks cholinergic action, is without effect until 20 days of age. Moreover, these effects do not appear to involve peripheral cholinergic action, since the quarternary congener of scopolamine (i.e. methyl-scopolamine), which does not pass into the brain, fails to affect activity levels (Anisman & Kokkinidis, 1974; Campbell et al, 1969; Feigley, 1974). In addition, benztropine, a potent anticholinergic which resembles scopolamine both structurally and pharmacologically (Thornburg & Moore, 1973), produces similar results, suggesting that the observed effects are not specific to scopolamine alone. Confirmation and extension of these results have come from additional studies by Fibiger et al, (1970), in which it was observed that the typical antagonism of the amphetamine effects by pilocarpine (a cholinomimetic) was not apparent in rats younger than 20 days of age. Similarly, the synergism between d-amphetamine and scopolamine is not apparent at 10 days of age, but is present in the 30-day-old rat (McGeer et al, 1970). Clearly, it would appear that a balance exists between the catecholaminergic and cholinergic systems; however, the interaction between these transmitters

is not in effect until adequate maturation of the cholinergic system has taken place.

Greater specificity regarding catecholamine ontogenesis has come recently from studies attempting to divorce the actions of the dopamine and norepinephrine systems. Specifically, it has been reported (Lamprecht & Coyle, 1972) that the norepinephrine system develops somewhat slower than does the dopamine system. Although the enzymes involved in the conversion of dopa to dopamine (dopa decarboxylase) and dopamine to norepinephrine (dopamine-β-hydroxylase) progress equally quickly, the lag in norepinephrine appears to be related to a deficiency of vesicular storage. Further studies have suggested that both the norepinephrine and the dopamine systems are mature at day one of age, but may possibly differ in terms of the development of the receptor cells. Specifically, L-dopa, which increases dopamine and norepinephrine activity, produces behavioural excitation as early as one day following birth. Similarly, clonidine, a norepinephrine receptor stimulating agent, elicits a behavioural response similar to that seen with L-dopa. In contrast, responsivity to a dopamine receptor stimulating agent,apomorphine, is not observed until 3-4 days postpartum. Evidently, the production of both dopamine and norepinephrine differs by only a small amount, but the age at which the receptors mature varies by several days (Kellogg & Lundborg, 1972). It may well be the case that the catecholamine interaction with the forebrain cholinergic centres may involve both dopamine and norepinephrine. The precise specificity as to which is predominant i.e. dopamine or norepinephrine, is not clear since synthesis inhibition or depletion, as well as replenishment of either transmitter, have comparable effects (Campbell & Mabry, 1973).

Accordingly, both these amines may be equally important regarding potential interaction with acetylcholine. Summarizing then, the pharmacological approach to the understanding of the ontogenesis of the nervous system has yielded results comparable to that observed through autoradiographic and histological methods. Of equal importance, these data have also suggested that hyperactivity (or hyperreactivity) seen in immature animals may well be due to retarded cholinergic development. That is, when the cholinergic system is functional, the excitatory effects of the catecholamine system are dampened. However, so long as the inhibitory cholinergic system is not developed, activity levels are under control of the excitatory catecholamine systems. Thus, Campbell et al (1969) have postulated that the rapid rise in activity levels between 10 and 20 days of age is due to increased catecholamine activity, whereas the gradual decline in spontaneous activity following 20 days of age is due to the development of the inhibitory cholinergic system. This notion is discussed in greater detail in the following section.

Experimental-Clinical Implications

The concept of a noradrenergic-cholinergic model, with the noradrenergic system maturing more rapidly than the cholinergic system presents a viable framework for a theory of hyperactivity in the developing organism. In terms of human behaviour, it has been reported that severe hyperkinesis, as defined by chronic excessive levels of motor activity (Werry, 1968), has been estimated in at least 4% of all school-age children (Weiss, Minde, Douglas, Werry, & Sikes, 1967). This behavioural disorder has been referred to by a number of names including "hyperkinetic behaviour disorder",

"minimal brain dysfunction" (MBD), and "hyperactivity" (Grinspoon & Singer, 1973). The symptamology of the syndrome has been noted to include extreme overactivity, distractibility, short attention span, impulsiveness, marked mood fluctuations, and aggression (Eisenberg, 1972). The pharmacological and morphological evidence presented earlier on the differential development of the two systems associated with the motor activity suggests a possible link between biochemical development and the presence of a hyperkinetic syndrome. For example, the notion of delayed brain maturation is compatible with the empirical observation that hyperkinesis tends to improve with age (Millichap & Fowler, 1967). As such, it may well be the case that in the hyperkinetic child, there exists a retarded development of the cholinergic system, or overactivity of the catecholamine system. In fact, Wender (1974) has postulated that minimal brain dysfunction not only involves monoamine disturbances, but also genetic factors which may be responsible for these aberrant levels of neurotransmitters.

One disconcerting finding regarding the hyperkinetic syndrome is that administration of sympathomimetics such as amphetamine and ritalin, which in non-hyperkinetic children increase motility, result in a diminution of the hyperkinesis (Conners, 1970; Eisenberg, 1971; Millichap, 1968; Tec & Levy, 1971). Several possibilities exist which may account for the paradoxical effects of sympathomimetics in the hyperkinetic child. Specifically, where there exists an imbalance between noradrenergic and cholinergic activity, further increases in catecholamine action may result in a rebound involving yet a third system (e.g. serotonin) which inhibits the catecholamine effects (Swonger & Rech, 1972). Secondly, amphetamine

may produce end-product-inhibition, thus resulting in decreased synthesis of dopamine and norepinephrine (Cooper et al, 1970). Finally, amphetamine may result in release of catecholamines from storage granules, thereby leaving the organism with reduced levels of functionally effective amines (Welch & Welch, 1970). Such an effect would occur when utilization of amines exceeds total biosynthesis.

Turning again to the animal literature, several findings have been reported which are, in some respects, comparable to that seen in the human hyperkinetic syndrome. Specifically, it is well known that although activity increases with amphetamine, the extent of the increase is inversely proportional to baseline activity (Anisman et al, 1974; Fuller, 1970; Glick & Malloy, 1973; Mosser & Welch, 1973). Yet, in the experiments carried out to date, there has neither been a developmental approach to hyperkinesis in animals, nor has there been an attempt to employ animals exhibiting behaviour resembling hyperactivity. To this end, the present experiment represents an attempt to employ a possible animal analogue to the hyperactive syndrome seen in the developing human. For example, it has been observed that the C57BL/6 strain of mice exhibit a relatively low level of acetylcholine (Ebel, Hermetet, & Mandel, 1972; Pryor, Schlesinger, & Calhoun, 1966), as well as exceptionally low cognitive abilities (Anisman, 1974e). Moreover, general activity is greater in C57 mice than in the DBA strain, which in turn is greater than that of A mice (Anisman et al, 1974). It follows that the increased levels of activity seen in the C57 strain might be a function of low levels of acetylcholine and a related ineffectiveness of the cholinergic inhibitory system. Injections of an anticholinergic, such as scopolamine, should result in increased levels of

activity in those animals where large reductions in the system are possible, but should be relatively ineffective in animals with already reduced cholinergic levels. This hypothesis has in fact been supported, in that scopolamine has been shown to decrease levels of activity in the C57 strain (Abeelen & Strijbosch, 1969; Abeelen et al, 1970, 1971; Anisman et al, 1974; Oliverio, Eleftheriou, & Bailey, 1973). In contrast, d-amphetamine, a noradrenergic agent, uniformly increases activity levels in all three strains; however, this increase is inversely related to baseline activity levels.

A second point of interest in studies involving C57 mice is that this strain undergoes what has been termed a "popcorn" stage of activity at approximately 21 days of age (Anisman, unpublished observations; Wahlsten, unpublished observations). This period is evidenced by heightened locomotor activity and exaggerated reactions to novel stimuli, after which activity decreases; furthermore, this increase coincides with increased levels of norepinephrine in these animals (Schlesinger & Griek, 1970). However, in other strains of mice (i.e. A and DBA), this rise in locomotor activity is not observed, and they also exhibit lower levels of norepinephrine during this period of growth. It may well be the case that the "popcorn" stage of development is one in which the noradrenergic and cholinergic systems are at greatest imbalance, thus leading to the observed hyperkinesis. Accordingly, it may be possible to promote a retardation of activity levels at this age via drugs which at other times in development would augment motility.

Given the apparent congruency between the human hyperkinetic syndrome and that seen in mice, it would seem that a study involving genetic and ontogenic variables in response to pharmacological treatments might be a viable approach to the understanding of the etiology of human hyperkinesis. The present study represents an attempt to further investigate the noradrenergic and cholinergic developmental sequence in three strains of mice. Since the noradrenergic and cholinergic mechanisms differ in their rate of maturity (Campbell et al, 1960; Feigley, 1974; Fibiger et al, 1970), examination of drug effects at different ages presents an opportunity to determine whether observed increases in locomotor activity are a function of increased noradrenergic levels or initial low levels of cholinergic action. Since amphetamine is known to act upon the noradrenergic system (Houser & Van Hart, 1973; Javoy, Thierry, Kety, & Glowinski, 1968; Moore & Lariviere, 1963; Schildkraut & Kety, 1967; Taylor & Snyder, 1970), it is possible to administer such a drug at different ages postnatally to evaluate the maturation of this system. The use of the dextro-isomer of amphetamine (d-amphetamine) rather than the levo-isomer (l-amphetamine) is indicated by evidence that the former is a markedly more potent excitator of norepinephrine, while the levo-isomer is equipotent to the dextro-isomer in modifying dopamine action. Behaviourally, it has been shown that d-amphetamine is 10 times as potent as l-amphetamine in enhancing locomotor activity (Taylor & Snyder, 1970). By the same token, scopolamine can be used in a similar fashion to assess cholinergic development because of its associated effects on the cholinergic mechanism. For example, if this system develops at different rates in the three strains, one should

expect scopolamine to act only on those strains and at those particular ages where the cholinergic system is developed at the time of drug administration (Campbell et al, 1969). It is important to note that different dosages of each drug are suggested by several findings. Alhava and Mattila (1973), for example, report dose dependent differences in catecholamine levels in the brain and heart of mice measured at three ages postnatally following injection of amphetamine. Similarly, in a review of cholinergic actions, Carlton (1963) emphasizes the need of exeprimental evidence for different drugs in terms of a wider range of dosages and a variety of behavioural tasks. In fact, Anisman et al, (1974) have shown clear differences in dose response curves in different strains of mice, probably owing to variations in endogenous levels of acetylcholine, as well as in rate at which drugs are metabolized.

Finally, the drug and strain interactions are evaluated on general locomotor activity. This particular measure was employed because of its sensitivity to pharmacological treatments. Unlike tasks involving a learning or memory component, drug effects are not inhibited by overriding cognitive factors which are often observed in aversively or appetitively motivated tasks (Anisman, 1974e;Longo, 1966).

Summarizing, in the present experiment, an attempt was made to (a) outline the behavioural effects of d-amphetamine and scopolamine in three strains of mice, (b) infer the ontogenesis of catecholamine and acetylcholine systems from the response to pharmacological treatments, (c) evaluate strain-specific differences in the ontogeny of these two transmitter systems, and finally (d) determine whether relationships exist between

ontogenic and genetic variation in activity with the effectiveness of pharmacological treatments.

The strains employed were A/J, DBA/2J, and C57BL/6J mice. These strains were used because they represent a continuum of activity, with A mice exhibiting lowest levels of activity as adults, while C57 shows the highest levels of activity (Anisman et al, 1974). Moreover, these strains also show strain-specific variations in levels of acetylcholine and catecholamines. Specifically, C57 exhibits the lowest levels of acetylcholine and A the highest, whereas with respect to catecholamine activity, A mice exhibit low turnover rates (Bovet & Oliverio, 1973). Parenthetically, the within strain variability in the development of various fiber tracts in these strains is exceptionally small (Wahlsten, 1974a), as is the variation in response to drug treatments (Bovet & Oliverio, 1973). In fact, it has been suggested that the effects of scopolamine may be modulated by a single major gene (Sco) (Oliverio et al, 1973). Clearly, isogenic lines may be of great benefit in evaluating drug effects owing to the relatively small variation seen in the development of fibre tracts and transmitter systems. Mice were tested at either 14, 21, or 28 days postpartum. These ages were selected because motor coordination is fairly well developed at these times (Wahlsten, 1974b), and in addition, substantial data exists regarding catecholamine and acetylcholine levels at these particular ages (Schlesinger & Griek, 1970). Finally, the dosages of d-amphetamine and scopolamine employed were based on earlier studies (Anisman & Cygan, 1974; Anisman & Kokkinidis, 1974; Anisman et al, 1974), indicating that these were optimal levels. Additionally, lower dosages than the optimal were also used in the event of

the blood-brain-barrier being immature at these early ages; thus, extreme dosage effects could be determined. The design of the present experiment was therefore a 3(strain) x 3(age) x 6(drug treatment) factorial in which all factors were considered between groups.

Method

Subjects

Subjects were 486 experimentally naive mice represented equally from each of three highly inbred strains (A/J, DBA/2J and C57BL/6J). Mice were bred at the University of Waterloo from stock originally procured from the Jackson Laboratory, Bar Harbor, Maine. Females were removed when visibly pregnant from their mating cages (16-18 days) and housed individually. Offspring were tested at 14, 21 or 28 days, having spent their neonatal periods with the maternal parent. Entire litters were tested on the same day in order to eliminate potential confounds owing to removal of one or more offspring from the breeding cage and thereby affecting the rest of the litter (Wahlsten, 1974). Throughout the period between birth and testing, ad libitum access to food, water and maternal parent was available.

Apparatus

The testing apparatus consisted of a 21 x 21 x 30 cm. clear Plexiglas chamber. Photoelectric cells were situated on two walls 1.0 cm. above the floor and spaced 7 cm. apart, thus forming a 3 x 3 matrix. Crossing a photobeam resulted in a count being recorded. Once a beam was broken, this particular cell could not be activated again until a second beam was broken, thereby eliminating counts due to head bobbing or tremor. Activity counts were recorded on a series of digital counters.

Procedure

At the appropriate age (14, 21 or 28 days), mice were individually removed from the parent cage and treated with d-amphetamine sulphate

(0.5, 1.0, or 5.0 mg/kg), scopolamine hydrobromide (0.5 or 1.0 mg/kg) or physiological saline (2 ml/kg) (n=9/cell). All drugs were dissolved in bacteriostatic water in a volume of 0.5 mg/ml. Mice were placed in a holding cage for a ten minute period, after which they were put into the test chamber for ten minutes. During this time, activity was recorded.

Results

The number of photocell crossings were transformed through the $\sqrt{x + 1}$ in order to reduce heterogeneity of variance. The mean (± standard error) of the transformed scores are depicted in Figure 1 as a function of Strain, Age and Drug Treatment. Analysis of variance of the transformed

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Insert Figure 1 about here

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activity scores yielded a significant Strain x Age x Drug Treatment interaction ($F=3.79$, $df=20/432$, $p<.01$) (see Appendix A, Table 1). Subsequent Newman Keuls multiple comparisons ($\alpha=.05$) were carried out on the simple main effects involved in this interaction, (see Appendix A, Table 3) the only exception to this being the case of saline treated animals. Inspection of the data had revealed an exceptionally large means-variance relationship which, owing to the extreme variance of amphetamine (5 mg/kg) groups, was not significantly reduced through the transformation. Indeed, the error variance of the overall analysis was greatly inflated by the drug groups, thus obscuring potential differences in saline animals whose activity levels and variance were relatively low. Accordingly, a separate analysis of variance of the activity scores for the saline treated animals was performed. This analysis revealed a Strain x Age interaction ($F = 6.23$, $df = 4/72$, $p<.01$) (see Appendix A, Table 2). This particular finding was

subsequently replicated (Anisman & Remington, unpublished report). In any event, Newman Keuls multiple comparisons among the saline treated animals revealed that in all three strains, activity was greater among 21- and 28-day-old mice than that witnessed in 14-day-old mice (see Appendix A, Table 4). The actual level of increase varied across the three strains. Specifically, at 14 days of age, DBA mice were significantly more active than either A or C57 mice. The increase in C57 mice at 21 days of age was such that C57 did not differ from DBA, but both strains exhibited activity levels which exceeded that seen in A mice. At 28 days of age, a small but nonsignificant decline in activity was observed in DBA and C57 mice, such that C57 levels of activity, but not DBA activity, exceeded that observed in A mice. The decline in activity between 21 and 28 days of age is consistent with numerous other reports indicating that activity reaches maximal levels at 21 days, after which a decline is noted toward 28 days, when activity begins to resemble that seen in adult animals (Campbell et al, 1969; Fibiger et al, 1970; Himwich, 1970; Mabry & Campbell, 1974; Moorcroft et al, 1971; Thornburg & Moore, 1973). Indeed, among adult mice, it is typically observed that C57 is considerably more active than either DBA or A. Thus, it is not unlikely that if activity had been monitored at additional ages, the decline in activity would have been more pronounced.

Turning to the effects of d-amphetamine and scopolamine, it was observed that at the 5mg/kg dosage, d-amphetamine enhanced activity in all strains as early as 14 days of age. In DBA, this effect did not reach

a significant value, as it did in A and C57 mice. Nevertheless, effects of d-amphetamine were maintained at 21 and at 28 days of age. It is interesting to note, though, that at 14 days of age, the most pronounced amphetamine effect was seen in C57 mice, whereas at 21 and 28 days of age, amphetamine has substantially greater effects in A mice than in DBA or C57. The latter drug effect is precisely the same as that observed in adults of these strains. Evidently, although amphetamine alters activity as early as 14 days postnatally, the response is not comparable to that of adult levels. Quite possibly, maturation of the catecholamine system continues after 14 days of age, or the effects of the drug are modified with the maturation of ancillary systems which modify the effects of central catecholamine agents (see, for example, Swonger & Rech, 1972). A second interesting finding here is that at 21 days of age, where C57 exhibits maximal levels of activity, the response to amphetamine is at its lowest level and does not reach statistical significance. Essentially, such a finding suggests that although the catecholamine system is intact (witness the 14 day response to amphetamine), the effectiveness of amphetamine in increasing activity is limited by developmental factors related to endogenous catecholamine levels, turnover, degradation, or possibly involvement of a second antagonistic system.

The response to scopolamine was found to be very different from that of amphetamine. Specifically, at 14 days of age, scopolamine did not increase activity in any of the strains. In fact, a small but nonsignificant decline in activity was seen in DBA mice. However, neither A or C57 mice exhibited any change in behaviour in response to the drug treatment. By

21 days postnatally, a pronounced increase in activity was seen in response to both doses of scopolamine with the DBA strain; in contrast, A and C57 animals revealed smaller increases which failed to reach significance. Finally, at 28 days of age, all three strains of mice exhibited increased activity levels in response to scopolamine. Quite clearly, scopolamine will exert disinhibitory effects on activity in all strains; however, the appearance of these effects becomes apparent much later than the amphetamine effect. In addition, the disinhibitory influence interacts with strain in terms of the age at which disinhibition is observed.

It is important to note that the results presented in this paper have dealt exclusively with general activity. Additional data regarding reaction to a startle stimulus i.e., air puff, have revealed precisely comparable results (Remington and Anisman, unpublished report), suggesting that the drug effects observed here deal with general activity and reactivity.

Discussion

Consistent with earlier reports (Anisman, 1974b,c; Anisman et al, 1974), strain differences in activity levels were observed; however, the present data indicated that these strain differences interacted with age. At 14 days postnatally, C57 mice exhibited minimal levels of activity relative to A and DBA mice. With increased development, the absolute rise in activity of C57 mice was such that it exceeded that observed in A mice and equalled that of DBA. Together with other reports involving full adult mice (Anisman, 1974; Anisman et al, 1974), it is apparent that activity in the C57 strain surpasses that seen in DBA.

Recently, Oliverio et al (1973) have reported that activity levels, although inherited in a polygenic fashion, are largely controlled by a single autosomal gene (Exa). The observed strain differences in activity with age suggest either epistatic interaction, or a gene effect which modulates behaviour only at later stages of development. In as much as activity also seems to involve neurochemical maturation (Campbell & Mabry, 1973; Carlton, 1963, 1969; Moorcroft et al, 1971), it may well be the case that the gene in question involves adequate neuronal transmission and/or maturation of enzymes associated with these transmitters. Indeed, while the effects exerted by this particular gene may be present, the manifestation of its behavioural consequences could be limited by differences in morphological development. For example, it is known that development of different brain structures vary across inbred strains and their various F_1 and F_2 hybrids (Wahlsten, 1974a, 1974b). This work indicated differences in development of areas associated with the limbic system, as well as

differences in growth of up to 71 different fibre tracts including both forebrain and hindbrain structures.

Turning to the drug effects observed in the present investigation, it was observed that regardless of strain, a large increase in activity was observed with administration of d-amphetamine. This particular finding is congruent with the many earlier reports indicating adequate and early maturation of the hindbrain structures (Eiduson, 1971; Jackson, 1931; Kim et al, 1970; Moorcroft, 1971). Since d-amphetamine produces increased synthesis, release, and reuptake of both dopamine and norepinephrine, it is difficult to ascertain whether dopaminergic and noradrenergic structures develop differentially. Further analysis involving enzyme inhibitors such as α-methyl-ρ-tyrosine or FLA-63 may thus be crucial for determining which of these systems is in fact the more important one in mediating the early effects of amphetamine (Kellogg & Lundborg, 1973). Nevertheless, there are substantial data indicating that dopamine, and possibly dopamine and norepinephrine modulate the effects of d-amphetamine. Thus, it would appear safe to assume that the former of the two transmitters (and possibly both) are necessary for the excitatory effects of amphetamine to become apparent. However, the important point of the present investigation was that the catecholamine system was sufficiently mature at 14 days of age to allow for the drug effect to be apparent.

It is of interest to note that at 14 days of age, d-amphetamine appears to have more potent effects on activity among C57 mice than among A or DBA mice. However, at 21 days of age, the full adult relationship is observed, in that A mice exhibit a more pronounced response to amphetamine than DBA

mice, which in turn exhibit a greater response than C57 mice (cf. Anisman, 1974c, 1974d). Parenthetically, the response to d-amphetamine is apparently not due to baseline levels of activity, since F_1 hybrids revealing high levels of activity, may exhibit large increments in activity with amphetamine treatment, whereas a cross resulting in equally high levels of activity may result in a small response to amphetamine. Thus, the actual effect of the drug is probably dependent upon neurotransmitter synthesis, release, reuptake, and degradation. However, the behavioural concomitants of the neurotransmitter status may be subject to interaction with other inhibitory transmitter systems (cf. Anisman, 1974c).

In any event, the fact that highest levels of catecholamines in C57 are seen at 21 days of age (Schlesinger & Griek, 1970), and that this particular age is the one at which the smallest amphetamine effects are observed, is consonant with the hyperactivity hypothesis posited earlier. Namely, when the relations between catecholamines and acetylcholine are not in balance, further increases in norepinephrine may result in a rebound effect, or end-product-inhibition, thereby decreasing activity levels. In as much as the amphetamine effect is apparent at 14 and 28 days, but to a lesser degree at 21 days may well be due to the imbalance between acetylcholine and catecholamines produced by the high levels of norepinephrine. Specifically, at neither 14 or 28 days of age is the balance in serious jeopardy, and consequently amphetamine augments activity levels. With the imbalance at 21 days, a physiologically mediated system protects the organism from further disturbances in the noradrenergic-cholinergic balance, thereby decreasing the excitatory effects of d-amphetamine. As

already suggested, it is not clear what the form of this prevention is, but rebound effects or end-product-inhibition are not unlikely candidates.

Turning to the effects of scopolamine, it was observed that in none of the strains was a behavioural change observed at 14 days postnatally. At 21 days of age, only DBA mice exhibited increased levels of activity, while at 28 days, scopolamine uniformly increased activity levels in all three strains. Quite clearly, response to amphetamine is manifested much earlier than to that of the anticholinergic, scopolamine. This finding is consistent with other reports indicating that manipulation of the noradrenergic system has profound effects on behaviour at an earlier age than alterations of the cholinergic system. Moreover, as the forebrain limbic structures are primarily cholinergic and the hypothalamus and midbrain structures are largely noradrenergic (Cooper et al, 1970), the temporal differences in the effects of amphetamine and scopolamine provide evidence for the caudal-rostral growth of the brain posited by Jackson (1931). However, the very fact that a differential response to scopolamine is noted across the three strains suggests that there are strain differences in terms of brain development, and in particular, cholinergic structures.

The notion that response to anticholinergics may be related to endogenous levels of transmitters has been supported by research carried out in several labs. Specifically, in those instances where low levels of choline acetyltransferase and acetylcholinesterase, two enzymes indicative of acetylcholine levels, have been observed i.e. in the C57 strain (Ebel, Hermetet, & Mandel, 1972; Mandel & Ebel, 1974), negligible responsivity to anticholinergic agents has been reported (Abeelen & Strijbosch, 1969;

Abeelen et al, 1970, 1971; Anisman et al, 1974; Oliverio et al, 1973). Conversely, where higher levels of enzymes associated with acetylcholine have been observed, as in DBA and A mice (Bovet & Oliverio, 1973; Mandel & Ebel, 1974), substantial increases in activity were recorded following treatment with scopolamine. Thus, it is not an unlikely possibility that a lack of response to scopolamine in immature animals simply reflects low levels of acetylcholine. With the maturation of the cholinergic system, the effectiveness of scopolamine in increasing activity becomes apparent. Of particular importance here is the fact that in A and C57 mice, the development of the cholinergic system is retarded to that of the DBA strain. Interestingly enough, although scopolamine has a pronounced disinhibitory effect in C57 mice at 28 days of age, these effects are eliminated or decline in adult animals suggesting further fluctuations of cholinergic activity with progressive development. In any event, it is significant that in C57 mice, a very pronounced increase in activity is observed between 14 and 21 days of age. This sizeable increase may well be due to the lack of an inhibitory system at 21 days of age, together with a very pronounced increase in levels of norepinephrine (Schlesinger & Griek, 1970). In effect, because of the gross imbalance between catecholamine and acetylcholine levels, administration of amphetamine may produce the apparently paradoxical effect of causing only minor increases in activity. In A mice, the cholinergic system is also not developed; however, because the catecholamine levels are not changing as drastically as in C57, the increase in general activity between 14 and 21 days of age is a moderate one. Furthermore, this notion may also account for the

fact that amphetamine produces a pronounced increase in activity at 21 days of age in A mice. Specifically, since norepinephrine levels are not as high as in C57, the noradrenergic-cholinergic balance is not seriously threatened, thus permitting the excitatory effects of amphetamine to become apparent. Moreover, with the development of the inhibitory cholinergic system by 28 days, the excitatory effects of amphetamine are dampened relative to that witnessed at 21 days of age.

It would seem reasonable that although catecholamine and acetylcholine modifications both affect behaviour independently, these systems also act in a coordinated fashion. As already suggested in earlier parts of this paper, these two systems might interact in a homeostatic type of process, whereby different excitatory and inhibitory systems antagonize each other. Similarly, independent modifications of the two systems may result in an additive, and even synergistic types of effects. For example, cholinomimetics such as pilocarpine have been found to reduce amphetamine induced excitation, while anticholinergics such as scopolamine potentiate this arousal (Campbell et al, 1969; Fibiger et al, 1970; Thornburg & Moore, 1973). The reversal appears to also be true, since administration of d-amphetamine results in decreased levels of acetylcholine (Vasko, Domino, & Domino, 1974). Moreover, these data also indicate that while a distinct balance exists between the excitatory catecholamine and inhibitory cholinergic systems, the cholinergic influence lags behind noradrenergic development during ontogenesis, a finding in agreement with the present data.

While it is clear that the catecholamine and acetylcholine levels modulate behaviour, it also seems to be the case that other putative transmitters are also involved, either directly or indirectly, in the proposed

balance model. Suggestions have been made concerning the role of the noradrenergic system in interaction with the serotonergic pathways. Specifically, para-chlorophenylalanine (ρ-CPA), a depletor of brain serotonin, interacts with amphetamine to increase activity levels in a synergistic fashion, while administration of 5-hydroxytryptophan (5-HTP) prevents this effect (Ellison & Bresler, 1974; Mabry & Campbell, 1973). Developmentally, it is of interest to note that depletion of serotonin by ρ-CPA fails to produce such synergistic effects with amphetamine at 10 days postnatally, although the effect is apparent by 15 days of age (Mabry & Campbell, 1974). Furthermore, lesions of the medial forebrain bundle result in dramatic reductions in telencephalic content of serotonin (60-84%), while at the same time producing a three-fold increase in amphetamine action (Green & Harvey, 1974).

In terms of dopaminergic pathways, it has been observed that dopamine receptor blocking agents, such as haloperidol, inhibit amphetamine induced increases in locomotor activity (Rolinski & Scheel-Kruger, 1973). In addition, both dopamine and norepinephrine appear to play a role in L-dopa induced activity increases (Maj, et al, 1971). Supporting the hypothesis that dopaminergic pathways are integral to noradrenergic increases in activity, are the findings that α-methyldopa, a substance which weakly stimulates dopamine receptors but has profound effects on norepinephrine, causes only slight increases in motor activity. If, however, it is given in combination with apomorphine, a dopamine receptor stimulating agent, substantial increases in motor activity are recorded (Anden, et al, 1973). Further evidence for the interaction of noradrenergic and dopaminergic

pathways stems from the recent literature on chemical lesions using 6-hydroxydopamine (6-OHDA). Administration of this substance sharply reduces brain levels of both norepinephrine and dopamine through destruction of their respective transmitter pathways (Ungerstedt, 1971, 1974; Masuoka & Alcaraz, 1973; Fibiger, Phillips, & Zis, 1973; Richardson, 1974). Following such lesions, L-dopa (the precursor of dopamine and norepinephrine), in combination with FLA-63 (a blocking agent of norepinephrine synthesis), results in increased locomotor activity (Ahlenius, 1974). Similarly, it has been reported that amphetamine-stimulated motor activity and stereotyped behaviour are reduced following treatments which decreased dopamine or dopamine and norepinephrine. However, such reductions are not seen in animals where only levels of brain norepinephrine are reduced (Hollister, Breese, & Cooper, 1974). Further investigations support the hypothesis that dopamine and noradrenaline act in a combined fashion in modulating spontaneous motor activity (Gordon & Shellenberger, 1973), as well as behaviours other than locomotion; for example, the interaction of these neurotransmitters has been related to stereotyped behaviours such as gnawing and continuous grooming (Molander & Randrup, 1974), in addition to alterations in body temperature (Caccia, Cecchetti, Garattini, & Jori, 1973).

The complexity of these interactions does not end here. Just as noradrenergic influences have been shown to interact with the cholinergic system (Carlton, 1963, 1969; Campbell et al, 1969; Fengley, 1974; Fibiger et al, 1970; Moorcroft et al, 1971; McGeer et al, 1971), it has been indicated that dopaminergic effects influence, at least in part,

acetylcholine. Following administration of 6-OHDA, reductions in acetylcholinesterase, the degrading enzyme for acetylcholine, have been reported (Consolo, Ladinsky, Samanin, & Ghezzi, 1974; Grewaal, Fibiger, & McGeer, 1974; Kim, 1973). In agreement with this data are findings that 6-OHDA lesions result in large increases in acetylcholine following administration of the drug (Grewaal et al, 1974; Kim, 1973).

Having observed these various interactions of neurotransmitter substances, it would seem reasonable that any pharmacological explanation of behaviour must involve a complex interaction of these various excitatory and inhibitory systems. In effect then, the suggestion here is that although catecholamine and acetylcholine levels serve to modulate both genetic and ontogenic variations in behaviour, other transmitter systems need to be evaluated to elucidate their role in modifying behaviour. With respect to phenomena such as the hyperactive syndrome, it may well be the case that the aberrant behaviours observed are due, in part, to acetylcholine and catecholamine imbalances, but in addition, other inhibitory systems such as serotonin may also be poorly developed. This is expecially important since increased norepinephrine and/or dopamine excitation are extensively magnified by decreases in serotonin (Ellison & Bresler, 1974; Green & Harvey, 1974; Mabry & Campbell, 1973, 1974). Thus, while variation in response to amphetamine and scopolamine may be indicative of retarded development, it is not sufficient to account for the entire hyperactive syndrome. Quite possibly, the hyperactivity and the inverse effects of sympathomimetics may be more apparent in strains or individual organisms suffering from a lag in serotonergic ontogeny. Further work to this end is currently underway.

Footnote

While acetylcholine is technically an excitatory transmitter in the sense that it induces neuronal activity, it is referred to as an inhibitory transmitter in the present document since it results in the reduction of ongoing locomotor behavior.

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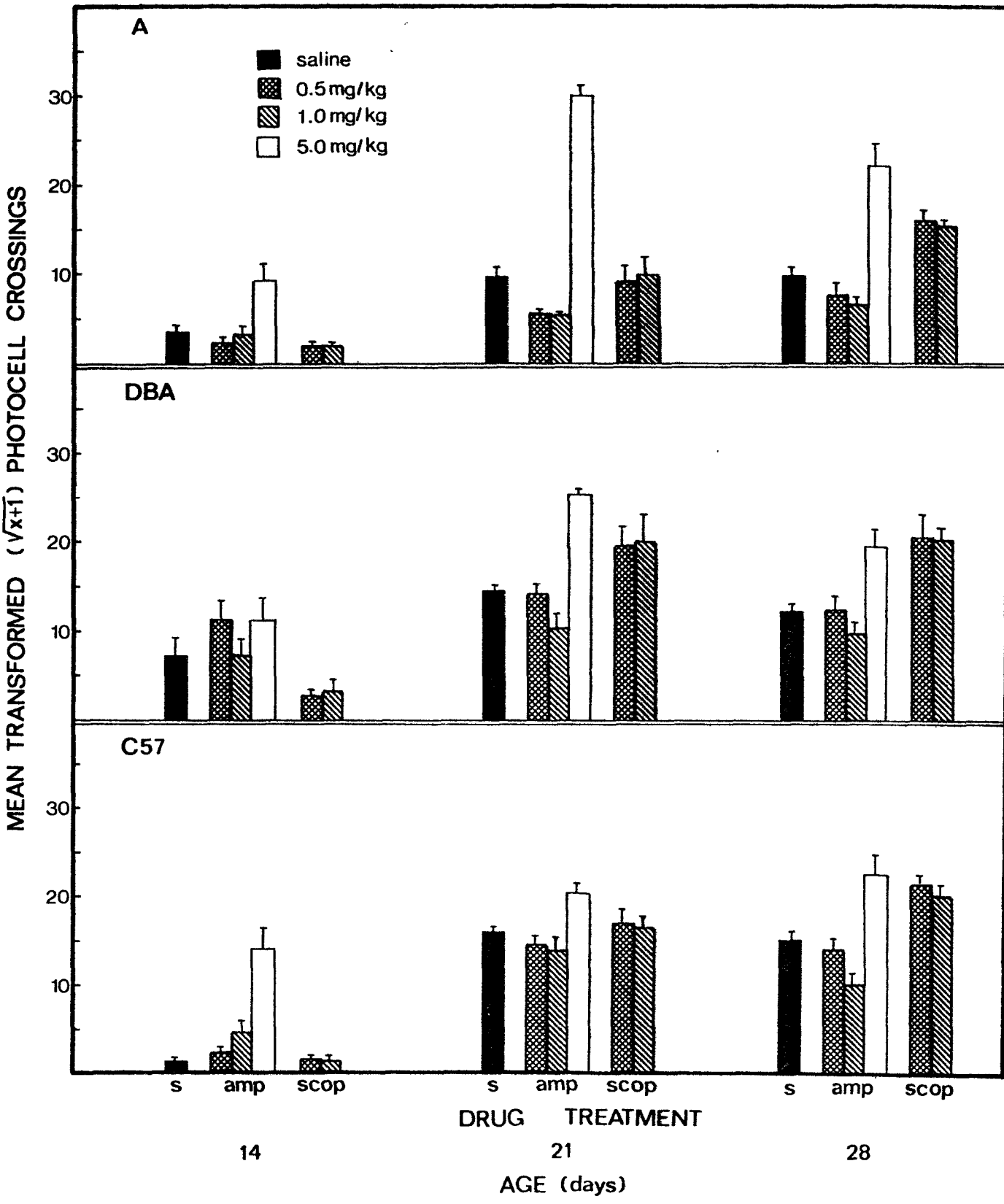
Figure 1. Mean transformed ($\sqrt{x + 1}$) number of photocell crossings ($\pm$ standard error of the mean) as a function of Strain, Age and Drug Treatment.


A
saline
0.5 mg/kg
1.0 mg/kg
5.0 mg/kg
DBA
C57
MEAN TRANSFORMED ($\sqrt{x+1}$) PHOTOCELL CROSSINGS
30
20
10
s
amp
scop
DRUG TREATMENT
14
21
28
AGE (days)

APPENDIX A

Table 1

Analysis of Variance of Transformed Photocell Crossings ($\sqrt{X+1}$)

as a Function of Strain, Age, and Drug Treatment

Between	df	MS	F
Strain (S)	2	770.62	42.61*
Age (A)	2	5714.7	315.99*
Drug (D)	5	1358.8	75.13*
S x A	4	121.16	6.70*
S x D	10	77.761	4.30*
A x D	10	249.68	13.81*
S x A x D	20	68.506	3.79*
Within Cells	432	18.085	
Total	485		

* p .01

Table 2

Analysis of Variance of Transformed Photocell Crossings ($\sqrt{X+1}$) for Saline Treated Animals as a Function of Strain and Age

Between	df	MS	F
Strain (S)	2	104.01	10.05*
Age (A)	2	720.39	69.58*
S x A	4	64.499	6.23*
Within Cells	72	10.35	
Total	80		

* p .01

Table 3

Newman-Keuls multiple comparisons of simple main effects on transformed scores ($\sqrt{X+1}$)

Sal: saline; A: amphetamine; S: scopolamine

A - 14 days

Drug Treatment	S(1.0)	S(0.5)	A(0.5)	A(1.0)	Sal	A(5.0)
S(1.0)	---	.01	.39	1.53	1.65	7.36*
S(0.5)		---	.38	1.52	1.64	7.35*
A(0.5)			---	1.14	1.26	6.97*
A(1.0)				---	.12	5.83*
Sal					---	5.71*
A(5.0)						---

* p .05

		r=2	r=3	r=4	r=5	r=6
	q.95 (r,432)	2.77	3.31	3.63	3.86	4.03
nMS	q.95 (r,432)	3.93	4.70	5.16	5.48	5.72

A - 21 days

Drug Treatment	Sal	A(0.5)	A(1.0)	A(5.0)	S(0.5)	S(1.0)
Sal	---	.32	3.72	4.65	4.78	24.73*
A(0.5)		---	3.4	4.33	4.46	24.41*
A(1.0)			---	.93	1.06	21.01*
A(5.0)				---	.13	20.08*
S(0.5)					---	19.93*
S(1.0)						---

* p .05

A - 28 days

Drug Treatment	A(1.0)	A(0.5)	Sal	S(1.0)	S(0.5)	A(5.0)
A(1.0)	---	1.3	3.44	8.80*	9.45*	15.78*
A(0.5)		---	2.14	7.50*	8.15*	14.48*
Sal			---	5.36*	6.01*	12.34*
S(1.0)				---	.65	6.98*
S(0.5)					---	6.33*
A(5.0)						---

* p .05

DBA - 14 days

Drug Treatment	S(0.5)	S(1.0)	A(1.0)	Sal	A(0.5)	A(5.0)
S(0.5)	---	.89	4.73	4.79	8.73*	8.81*
S(1.0)		---	3.84	3.90	7.84*	7.92*
A(1.0)			---	.06	4.0	4.08
Sal				---	3.94	4.02
A(0.5)					---	.08
A(5.0)						---

* p .05

DBA - 21 days

Drug Treatment	A(1.0)	A(0.5)	Sal	S(0.5)	S(1.0)	A(5.0)
A(1.0)	---	3.67	4.34	9.17*	9.93*	15.11*
A(0.5)		---	.67	5.50*	8.19*	11.14*
Sal			---	4.83*	7.52*	10.77*
S(0.5)				---	2.69	5.94
S(1.0)					---	3.25
A(5.0)						---

* p .05

DBA - 28 days

Drug Treatment	A(1.0)	Sal	A(0.5)	A(5.0)	S(1.0)	S(0.5)
A(1.0)	---	2.60	2.78	9.88*	10.63*	10.77*
Sal		---	.18	7.28*	8.03*	8.17*
A(0.5)			---	7.10*	7.85*	7.99*
A(5.0)				---	.75	.89
S(1.0)					---	.14
S(0.5)						---

* p .05

C57 - 14 days

Drug Treatment	S(1.0)	Sal	S(0.5)	A(0.5)	A(1.0)	A(5.0)
S(1.0)	---	.15	.29	1.06	3.21	13.12*
Sal		---	.14	.91	3.06	12.97*
S(0.5)			---	.77	2.92	12.83*
A(0.5)				---	2.15	12.06*
A(1.0)					---	9.91*
A(5.0)						---

* p .05

C57 - 21 days

Drug Treatment	A(1.0)	A(0.5)	Sal	S(1.0)	S(0.5)	A(5.0)
A(1.0)	---	.92	2.30	2.98	3.45	6.95*
A(0.5)		---	1.38	2.06	2.63	6.03*
Sal			---	.68	1.15	4.65
S(1.0)				---	.47	3.97
S(0.5)					---	3.50
A(5.0)						---

* p .05

C57 - 28 days

Drug Treatment	A(1.0)	A(0.5)	Sal	S(1.0)	S(0.5)	A(5.0)
A(1.0)	---	3.99*	5.08*	10.22*	11.65*	12.57*
A(0.5)		---	1.09	6.23*	7.66*	8.58*
Sal			---	5.14*	6.57*	7.49*
S(1.0)				---	1.43	2.35
S(0.5)					---	.92
A(5.0)						---

* p .05

Table 4

Newman Keuls multiple comparisons on transformed scores ($\sqrt{X+1}$) for Saline Treated Animals

14 days

Strain	C57	A	DBA
C57	---	2.03	5.06*
A		---	3.83*
DBA			---

* p .05

		r=2	r=3
	q.95 (r,432)	2.77	3.31
nMS	q.95 (r,432)	2.96	3.54

21 days

Strain	A	DBA	C57
A	---	4.76*	6.08*
DBA		---	1.32
C57			---

* p .05

28 days

Strain	A	DBA	C57
A	---	2.34	5.21*
DBA		---	2.87
C57			---

* p .05

A strain

Age (days)	14	21	28
14	---	6.41*	6.47*
21		---	.06
28			---

* p .05

DBA strain

Age (days)	14	21	28
14	---	4.98*	7.34*
21		---	2.36
28			----

* p .05

C57 strain

Age (days)	14	28	21
14	---	13.71*	14.52*
28		---	.81
21			---

* p .05

APPENDIX B

Table 1

Total Number of Photocell Crossings for Individual Subjects

sa a Function of Age, Strain, and Drug Treatment

A - saline

14 days	21 days	28 days
0	215	37
23	86	37
35	161	137
56	39	139
0	72	166
6	133	30
0	14	139
15	129	138
12	117	141

A - amphetamine (0.5 mg/kg)

14 days	21 days	28 days
0	19	19
5	29	16
17	18	147
0	69	41
0	68	30
0	36	154
0	41	105
51	29	25
1	1	98

A - amphetamine (1.0 mg/kg)

14 days	21 days	28 days
0	45	61
1	33	52
80	7	27
14	70	84
2	48	24
6	37	117
25	30	53
4	6	18
12	4	2

A - amphetamine (5.0 mg/kg)

14 days	21 days	28 days
47	1316	915
362	850	53
249	879	461
271	1053	660
19	1152	463
9	571	716
5	899	802
130	826	220
10	668	603

A - scopolamine (0.5 mg/kg)

14 days	21 days	28 days
3	99	112
2	2	133
18	29	231
0	136	319
0	164	360
0	383	190
0	84	447
11	11	257
1	47	345

A - scopolamine (1.0 mg/kg)

14 days	21 days	28 days
0	78	172
15	18	164
8	230	152
0	29	183
2	533	286
0	123	298
0	79	253
6	23	499
0	71	186

DBA - saline

14 days	21 days	28 days
7	120	136
0	261	204
2	335	72
330	71	179
60	225	254
85	197	54
182	193	160
107	255	162
1	368	198

DBA - amphetamine (0.5 mg/kg)

14 days	21 days	28 days
79	318	61
23	259	395
243	252	223
292	211	53
35	114	121
7	61	224
419	392	97
403	92	296
30	186	86

DBA - amphetamine (1.0 mg/kg)

14 days	21 days	28 days
1	69	27
0	16	76
126	189	20
115	130	306
19	52	145
177	84	95
4	31	137
292	423	54
15	159	106

DBA - amphetamine (5.0 mg/kg)

14 days	21 Days	28 days
116	633	396
0	771	343
64	803	392
3	738	471
309	672	76
289	561	690
105	413	633
262	695	568
371	588	152

DBA - scopolamine (0.5 mg/kg)

14 days	21 days	28 days
0	507	91
2	39	679
1	565	42
10	192	727
0	153	598
50	522	467
0	612	623
1	614	500
24	591	475

DBA - scopolamine (1.0 mg/kg)

14 days	21 days	28 days
0	724	546
0	590	255
0	511	209
7	358	542
9	551	601
211	227	237
0	453	356
22	573	595
2	538	518

C57 - saline

14 days	21 days	28 days
2	115	162
4	222	251
1	311	179
6	364	233
0	207	316
0	326	245
1	208	142
0	357	310
0	290	266

C57 - amphetamine (0.5mg/kg)

14 days	21 days	28 days
1	168	83
13	112	129
0	191	174
4	360	394
1	311	192
0	224	319
1	97	185
12	345	82
32	196	350

C57 - amphetamine (1.0 mg/kg)

14 days	21 days	28 days
3	108	117
131	119	147
0	437	155
74	312	181
2	4	31
2	348	111
56	281	3
33	150	32
0	178	325

C57 - amphetamine (5.0 mg/kg)

14 days	21 days	28 days
271	344	482
74	421	646
50	501	504
570	364	626
409	651	79
357	254	576
23	369	1161
231	417	490
141	581	402

C57 - scopolamine (0.5 mg/kg)

14 days	21 days	28 days
3	345	221
2	264	500
2	250	563
1	219	541
0	64	531
4	269	647
1	372	418
2	638	422
1	392	485

C57 - scopolamine (1.0 mg/kg)

14 days	21 days	28 days
1	192	195
1	304	356
2	208	549
0	457	604
0	298	474
0	119	239
1	293	490
2	393	418
1	314	493

PRIMARY PATHWAY IN THE SYNTHESIS OF CATECHOLAMINES

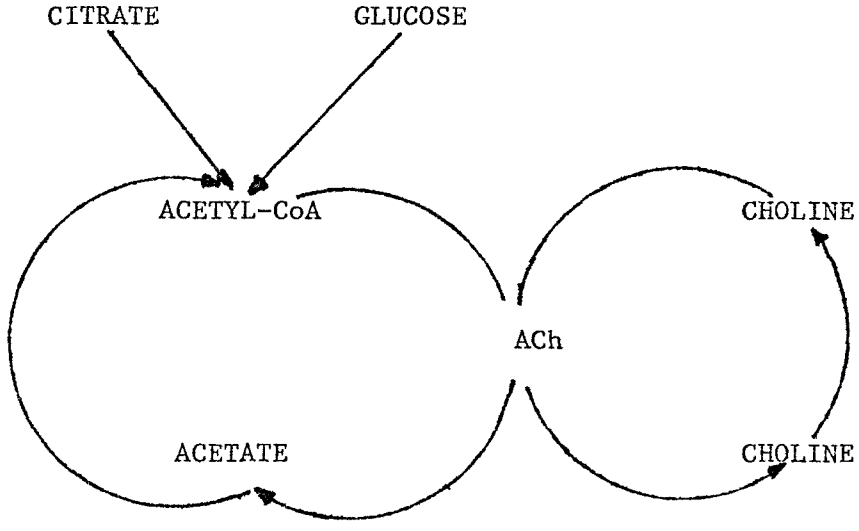
Tyrosine ----▶ (tyrosine hydroxylase)----▶Dopa ----▶(dopa decarboxylase) ----▶ Dopamine ----▶

(dopamine-B- hydoxylase) ----▶ Norepinephrine ---▶(phenylethanolamine-N-methyl transferase) ----▶

Epinepherine.

Note: enzymes associated with oxidation or hydrolysis are indicated in brackets. Catecholamines are degraded intracellularly by monoamine oxidase (MAO), and extracellularly by catechol-O-methyl transferase (COMT). (From Cooper, Bloom & Roth, 1970).

LATER STAGES OF SYNTHESIS AND METABOLISM OF ACETYLCHOLINE



(From Cooper, Bloom & Roth, 1970)