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Ontogeny of Muscarinic Cholinergic Supersensitivity in the Flinders Sensitive Line Rat

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DAWS, L. C. AND D. H. OVERSTREET. *Ontogeny of muscarinic cholinergic supersensitivity in the Flinders Sensitive Line Rat.* PHARMACOL BIOCHEM BEHAV **62**(2) 367–380, 1999.—The present study examined the ontogeny of muscarinic sensitivity in the Flinders Sensitive Line (FSL) rat, a model for human depression that was selectively bred for increased cholinergic function. In most cases, the FSL rats were more sensitive to the muscarinic agonists, oxotremorine and oxotremorine-M, early postnatally [13 days postpartum (P13)], suggesting that muscarinic supersensitivity is an inherent characteristic of FSL rats. The emergence of increased sensitivity to muscarinic agonists in FSL rats did not correlate with either the emergence of subsensitivity to the muscarinic antagonist, scopolamine, at P60 or with increased muscarinic (M1 or M2) receptor density. Relative to FRL rats, FSL rats did not exhibit increases in muscarinic receptor binding until P32 in the striatum and hippocampus and P120 in the hypothalamus. These results are consistent with the suggestions that (a) muscarinic supersensitivity, which appears early in development, may be associated with depressive disorders, and (b) the differences in muscarinic sensitivity early postnatally cannot be accounted for by an increase in the number of muscarinic receptors, per se. © 1999 Elsevier Science Inc.

FSL rats Muscarinic receptors Oxotremorine Scopolamine Core body temperature Locomotor activity Depression

SINCE Janowsky and co-workers proposed the cholinergic–adrenergic hypothesis of mania and depression in 1972 (27), considerable evidence has accumulated supporting a role for muscarinic cholinergic involvement in the etiology of affective disorders (30,31). Individuals with depressive disorders have been reported to be more sensitive to the behavioral and physiological effects of muscarinic cholinergic agonists than are normals (5,28,40,55). There is, though, some uncertainty whether this "cholinergic supersensitivity" is a cause or a consequence of the depressive disorder. For example, Berger and co-workers (5) found that supersensitivity to cholinergic agonists only occurred when depressive symptomatolgy was present, whereas Sitaram et al. (62) reported that a heightened response to muscarinic agonists in a euthymic individual was a predictor of a history of depressive disorders. This apparent discrepancy may be attributed, at least in part, to the populations used. The former used primarily unipolar patients, while the latter used a majority of bipolar depressive, which are recognized to have a higher genetic loading (22). If cholinergic supersensitivity is a biological trait underlying at least the bipolar disorder, then children of bipolar parents should exhibit a supersensitive response to muscarinic agonists. Only recently have pharmacological studies of children genetically at risk for depressive disorders emerged. For example, Schreiber and co-workers (60) reported that rapid eye movement (REM) sleep shortening induced by the long-acting cholinergic agonist, RS 86, was much greater in probands at risk for major depression than in normals. Whether muscarinic supersensitivity in young individuals is predictive of later depressive episodes is yet to be determined.

One way to address the hypothesis that cholinergic supersensitivity is a trait marker for depressive disorders is to use

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an animal model genetically bred for increased muscarinic cholinergic sensitivity. The Flinders Sensitive Line (FSL) rats were originally selected on the basis of the weighted average of three responses (change in core body temperature, body weight, and operant responding for a water reward) to the anticholinesterase, diisopropyl fluorophosphate (DFP) (42,57). The FSL rats had a greater sensitivity to these responses, in particular, change in core body temperature, than the control, Flinders Resistant Line (FRL) rats (42,57). Later, when it became clear that the FSL rats were also sensitive to muscarinic agonists (43) and had elevated numbers of muscarinic receptors (18,44), further maintenance of these lines was based on their hypothermic response to the short-acting muscarinic agonist, oxotremorine (46). Extensive studies have demonstrated that the FSL rats meet valid and reliable criteria as an animal model for depressive disorders [for review, see (46,48)], and because of the behavioral symptomatology they exhibit as well as anti-immobility responses to therapeutic drugs [see (48)], they may more closely model major depression. Analogous to studies in children of affectively ill parents, the FSL rats also exhibit muscarinic supersensitivity at the youngest age tested [21 days postpartum (P21), i.e., well before rats have fully matured] (18). Thus, cholinergic supersensitivity in FSL rats may be "innate," and occur before any significant integration of the muscarinic cholinergic system with other neuronal systems.

The underlying neurochemical bases for the early emergence of this supersensitivity remain unresolved. One possibility is an increase in central muscarinic acetylcholine receptors (mAChR). To date, the only data relating to mAChR density in humans that may be related to depression is that derived from individuals who committed suicide (i.e., assumed to be depressed). By and large, these studies have revealed no difference in cortical mAChR density between tissue derived from individuals who committed suicide and control subjects (32,37,63). However, because the brain region(s) responsible for mediating the exaggerated muscarinic responses in depressives is unknown, it is possible that brain regions other than the cortex, for example, those comprising the limbic system, may express an increased density of mAChRs. Analogous to studies in humans, adult FSL and FRL rats do not differ in total cortical mAChR density. However, adult FSL rats exhibit 20% more [^{3}H]-quinuclidynl benzilate ([^{3}H]-QNB) binding sites in both the striatum and hippocampus relative to the FRL rats (18,44,51). A developmental study of muscarinic-induced behavioral sensitivity and mAChR density in FSL rats may be heuristic in determining whether muscarinic cholinergic supersensitivity is a trait predisposing to depressive phenomenology. If hyperfunction of the central muscarinic cholinergic system is a trait, as opposed to a state marker of endogenous depressive disorders [e.g., (29,45)], it is hypothesized that neonatally an increase in mAChR number and/or in behavioral indices of cholinergic function should be found in the FSL rats compared to control FRL rats.

Preliminary receptor binding studies using brain tissue derived from FSL and FRL rats aged P19 and P61, revealed a higher density of striatal mAChR in FSL rats than in FRL rats aged P61, but no differences in the receptor density of younger FSL and FRL rats (18). Although this finding argues against the proposed early changes in mAChR, behavioral and physiological changes did appear early, suggesting that these indices need not be directly associated (18). Alternatively, because [^{3}H]-QNB is a relatively nonselective mAChR ligand in that it does not differentiate between the pharmacologically distinct mAChR subtypes, it is possible that changes in one or more of the mAChR subtypes may be masked when total mAChR is quantified.

To this end, the present investigation aimed to confirm and extend the results of preliminary studies by correlating the ontogeny of behavioral and physiological sensitivity to the muscarinic agonist, oxotremorine, and antagonist, scopolamine, with that of central M1 and M2 receptor density in rats aged P13 to P120.

METHOD

Animals

FSL and FRL male and females rats were from the 40th to 49th generations bred and maintained in the School of Biological Sciences, The Flinders University of South Australia. Each new generation of rats was screened for behavioral sensitivity to muscarinic agonists and the subsequent generation of FSL rats obtained by mating the 10 most "sensitive" males and females, while the 10 most "resistant" males and females were mated to produce the next generation of FRL rats. Breeding pairs were matched such that no brother–sister matings occurred. It should be noted that although inbreeding was minimized; this inevitably has occurred due to the small size of the initial colony (46). Pups were housed with their respective dams until weaning (P32), after which time they were housed in groups of six to eight. Rats from each litter were randomly assigned to home cages and treatment groups in order to minimize any litter effects. All animals were maintained under conditions of constant temperature (22 $\pm$ 1°C) and 50% humidity. Room lighting was on a 12–12 light–dark cycle, with lights on at 0700 h. All experiments involving rat handling were performed between 0800 and 1300 h. Food and water were provided ad lib. All experimental protocols were approved by the Institutional Review Committee for the use of Animal Subjects.

Core Body Temperature Recording

Core body temperature was recorded by inserting a lubricated thermocouple probe (Eirelec 5000 hand-held thermometer) 1–5 cm into the rectum (based on the age and size of the rat). Temperature was recorded to the nearest 0.1°C and was usually stable within 15 s of insertion of the probe. Baseline temperatures were obtained 1 to 2 h prior to drug administration. The data are expressed as mean $\pm$ SEM temperature change from baseline, with each animal serving as its own control.

Locomotor Activity

Spontaneous locomotor activity was measured by placing the animal in the center of a Perspex chamber (30 $\times$ 60 $\times$ 30 cm), with a grid ruled on the floor. For rat pups and juveniles ($\leq$P45) the grid divisions were 5 $\times$ 5 cm, and for adults (>P45) they were 10 $\times$ 10 cm.

Each rat was allowed 15 s to habituate to the chamber surroundings. Line-crossing activity was then recorded with hand-held counters. A line crossing was scored when the animal's forepaws completely crossed a line. The test duration was either 1 min (for agonist studies) or 5 min (for antagonist studies). Activity measures were always scored by the same observer to reduce errors caused by variations in experimenter subjectivity. The observer was blind to both the rat line and treatment. Baseline activity levels were obtained 2 h prior to challenge with drugs. Drug-induced changes in activity are expressed as mean $\pm$ SEM deviations from baseline or

mean ± SEM percent change from baseline where each subject served as its own control.

Drugs

Methyl atropine nitrate (MA), oxotremorine sesquifumarate (OXO), scopolamine hydrochloride (SCOP), and scopolamine methyl nitrate (SCOP-M) were purchased from the Sigma Chemical Company (St. Louis, MO), and oxotremorine-M (OXO-M) from Research Biomedical Incorporated (Natick, MA). All drugs were dissolved in isotonic saline on the morning of use and kept on ice until injection. Doses were based on the weight of the salt. All drugs were injected subcutaneously (SC) into the right flank of the rat.

Procedure

Sensitivity to muscarinic agonists: (a) Single dose studies. Animals were assigned to one of six treatment groups comprising OXO (0.25 μmol/kg) ± MA (0.73 mmol/kg), OXO-M (0.25 μmol/kg) ± MA (0.73 mmol/kg), MA (0.73 mmol/kg), or saline (SAL). OXO-M is a quaternary derivative of OXO and does not cross the blood–brain barrier (BBB) (53,58). MA does not cross the BBB, and was used to block the peripheral effects of OXO (26). Rats were rotated through each treatment to minimize the likelihood of drug tolerance. Each rat was used eight times, i.e., challenged at ages P10, P13, P15, P18, P21, P25, P30, and P50.

Rats were weighed and baseline core body temperatures obtained on the morning of the drug challenge tests. They were then injected with the appropriate drug combination and 30 min later core body temperature was measured.

(b) Dose–response studies. Based on the results of single dose studies using OXO and SCOP (see below), dose–response curves for OXO-induced changes in core body temperature and locomotor activity were constructed for rats aged P13, P21, P32, and P100. The doses of OXO were 0.15, 0.3, 0.6, and 0.8 μmol/kg for all ages tested with additional doses of 0.019, 0.038, and 0.075 μmol/kg included in the compilation of the dose–response curves for rats aged P100 where there were more rats available for use. A dose of 0.075 μmol/kg was given to a sample of rats at P13 to provide additional data relating to drug-induced changes in core body temperature and locomotor activity in young rat pups. All rats were given MA (0.73 mmol/kg) 15 min prior to OXO, with the exception of a subset of P13 rats, which received SAL prior to OXO. Locomotor activity and core body temperature were measured 15 and 30 min after the final injection. The rats received the doses in random order, with at least 8 days between tests, and no rat received the same dose more than once.

Dose–response curves for OXO-M were constructed for rats aged P18 and P100 only. The doses of OXO-M were 0.038, 0.075, 0.15, 0.3, and 0.6 μmol/kg. These rats received SAL 15 min prior to OXO-M.

Sensitivity to muscarinic antagonists. Male FSL and FRL rats were assigned to one of three treatment groups comprising SCOP (4 mg/kg), methylscopolamine (SCOP-M, 4 mg/kg) or SAL. SCOP-M is a quaternary derivative of SCOP and reputedly does not cross the BBB (26). The dose selected was based on dose–response studies by Campbell and co-workers (10), who showed that higher doses best delineate age-dependent changes in the response of rats to SCOP. In a post hoc experiment, a lower dose of SCOP (0.2 mg/kg) was given to a group of adults (P80) because sensitivity differences between the rat lines have been reported at lower doses (43). To minimize the possibility of drug tolerance, rats were rotated through each treatment so that each animal received the same drug once every three trials (i.e., the equivalent of every 15 days). Test days were separated by a minimum of 5 days to reduce the potential for habituation to the open field chamber.

SCOP does not exert any marked effect on core body temperature, so this parameter was not measured. Instead, the duration of observation of locomotor activity was increased to 5 min in an attempt to reduce the variability associated with this measure. Locomotor activity recordings commenced 30 min after injection when SCOP-induced hyperactivity is clearly apparent (43). Baseline data were not collected prior to the drug tests due to practical limitations, and also to avoid habituation to the open-field chamber on the test day. For this reason the locomotor activity of SAL-treated control rats served as the baseline for SCOP-M- and SCOP-treated rats.

Data Analysis

Data were submitted to either three-way analysis of variance (ANOVA), for each dose, where the main effects were age, line, and gender, or two-way ANOVA, where the main effects were combinations of the above. The probability level for significance was $p < 0.05$. The doses that elicited 50% of the maximal response (ED_{50}s) from dose–response studies were determined by nonlinear least squares regression (GraphPad Inplot, GraphPAD Software Inc, San Diego, CA) to a four-parameter logistic equation for a sigmoid curve. ED_{50}, baseline response (E_o), and maximal effect (E_{max}) were tested for significance using a *t*-test between two means (GraphPad Instat). Values for ED_{50} were subjected to log transformation before applying any parametric statistical test. Prior to any inferential analyses, data were tested for homogeneity of variance using Bartlett-Box F and Cochran's C-tests. Both tests confirmed homogeneity of variance.

Muscarinic Receptor Binding

Rats, not previously used in drug challenges, were selected at random from within a litter. To minimize litter effects, no more that two rats were taken from any one dam. Animals were sacrificed by decapitation and the brain rapidly removed and frozen on liquid nitrogen before being stored at −80°C. All sacrifices were performed between 0800 and 1200 h, and assays performed within 8 weeks of freezing. At the time of use, brains were thawed, and the cortex, striatum, hippocampus, and hypothalamus dissected out, weighed, and homogenized in ice-cold Na+/K+ phosphate buffer (50 mM, pH 7.4) in the presence of the protease inhibitor, phenylmethylsulfonyl fluoride (PMSF, 20 mM, Boehringer, Mannheim, Germany). The homogenates were subsequently dialyzed for 10 h using 25 mM Na+/K+ phosphate buffer (pH 7.4, flow rate 500 μl/s). Homogenates were diluted to yield final protein concentrations of approximately 0.2 mg/ml for the cortex, striatum, and hippocampus and 0.5 mg/ml for the hypothalamus.

Because the amount of tissue from an individual brain was insufficient for a reliable saturation assay, tissue from three rats (matched for line, age, and gender) were pooled. For saturation binding, the homogenates were incubated with increasing concentrations of [^{3}H]-QNB (specific activity 43.0 Ci/mmol, Dupont-NEN) ranging from 0.1 to 0.9 nM. Single-point binding assays, using a near saturating concentration of [^{3}H]-QNB (0.7 nM), were concurrently performed employing the individual homogenates that had contributed to the pooled tissue. Sufficient quantities of pooled homogenates were reserved for competition binding assays where [^{3}H]-QNB (0.7

nM) was incubated in the presence of increasing concentrations of unlabeled pirenzepine (PZ, 10^{-10} to 10^{-4} M), a selective M1 muscarinic antagonist (23,24,66).

Final assay volumes were 4.5 ml (saturation and single-point binding), comprising 4 ml, 50 mM Na+/K+ phosphate buffer and 500 μl of homogenate, or 5.0 ml for competition binding (4 ml, 22 mM Na+/K+ phosphate buffer, 500 μl of homogenate and 500 μl of PZ). Incubation of homogenate with ligand was for 2 h at 25°C. Bound ligand was isolated by rapid vacuum filtration on to Whatman GF/B filters using a Brandel M-24R receptor binding harvester. The reaction was terminated, and unbound ligand flushed by rapid repeated washing with ice-cold isotonic saline (3 × 5 ml washes). Scopolamine hydrochloride (5×10^{-6} M) was used to determine nonspecific binding. Radioactivity was determined using a Beckman LS-5800 scintillation counter (ca. 40% counting efficiency), and protein was determined by the method of Lowry et al. (36), with bovine serum albumin as the standard.

Specific binding data were submitted to unweighted nonlinear regression, and the saturation binding isotherm fitted according to the Michaelis-Menton equation. The maximal number of specific binding sites (B_{max}) for single-point [^{3}H]-QNB binding data was estimated according to the Michaelis-Menton equation, where the apparent dissociation constant (*K*d) used was calculated from the associated saturation isotherm. These data were subjected to two-way ANOVA, with line and age as the factors. Data for the inhibition of [^{3}H]-QNB binding by PZ were fitted to one- and two-site binding models according to the Law of Mass Action by weighted (reciprocal of the variance) nonlinear least-squares best fit (GraphPad Inplot). The concentration inhibiting 50% of maximal binding (IC_{50}) values were converted to inhibition constant (*K*i) values according to the Cheng-Prusoff equation (13,19).

RESULTS

Baseline Measures

There were age, $F(19, 2244) = 2571.64$, $p < 0.001$, gender, $F(1, 2244) = 1402.17$, $p < 0.001$, and line, $F(1, 2244) = 20.71$, $p < 0.001$, effects on the changes in body weight. Interaction effects were apparent between (a) age and line, $F(19, 2244) = 1.62$, $p < 0.05$, where both male and female FSL rats weighed more than their FRL counterparts through to P51 and less thereafter; and (b) gender and age, $F(19, 2244) = 99.64$, $p < 0.001$. The expected gender difference did not emerge until P31 and increased with age. Core body temperature increased with age, $F(19, 2244) = 208.96$, $p < 0.001$, and FSL rats had slightly, but significantly, higher baseline temperatures than their FRL counterparts [main effect line, $F(1, 2244) = 24.09$, $p < 0.001$]. Locomotor activity changed significantly with age, $F(19, 2244) = 17.20$, $p < 0.001$, gender, $F(1, 2244) = 66.56$, $p < 0.001$, and line, $F(1, 2244) = 59.34$, $p < 0.001$. There were significant interaction effects between (a) line and age, $F(19, 2244) = 6.26$, $p < 0.001$, where FRL rats exhibited a peak in activity at P21 before declining to adult levels, while FSL rats did not reach a peak in activity until P50, but unlike the FRL, rats maintained this relatively high level of activity over the remainder of the ages examined; and (b) gender and age, $F(19, 2244) = 1.74$, $p < 0.05$, where females became increasingly more active than males with age [see (17) for means and variance; (18)]. Because of these significant differences in baseline between genders and or lines, the analyses of drug effects were based on changes from baseline.

Development of Drug-Induced Changes in Core Body Temperature

Single-dose studies. No significant gender effects were established for any of the parameters referred to below; thus, within a line the following results represent pooled data for male and female rats (see following).

(a) Saline and methyl atropine controls: rats treated with SAL showed relatively small deviations from baseline core body temperature ranging from −0.9°C (at P13) to +0.14°C (at P30). There was no significant difference between FSL and FRL rats with respect to change in core body temperature exhibited after SAL. Rats treated with MA exhibited changes in core body temperature ranging from −1.4 to 0°C (i.e., no change from baseline). Across all ages tested, FSL rats generally showed a small, but significantly, greater decrease in core body temperature compared to their FRL counterpart [main effect line, $F(1, 181) = 3.93$, $p < 0.05$]. There was no significant effect of age on MA-induced changes in core body temperature [see (17)].

Comparing SAL and MA treatment groups, MA was found to generally produce hypothermia, whereas with SAL treatment, the rat's body temperature was generally within error of baseline. Thus, a significant treatment effect occurred, $F(1, 411) = 5.61$, $p < 0.05$. A small, but significant line difference was also revealed, $F(1, 411) = 4.03$, $p < 0.05$. When the analysis was applied to each rat line independently, it was found that MA produced a significantly greater hypothermia than SAL in FSL rats [FSL, $F(1, 232) = 38.55$, $p < 0.001$], but the change in core body temperature exhibited by FRL rats after SAL or MA was not different.

(b) Oxotremorine-M and oxotremorine (±MA): Fig. 1a illustrates age-dependent changes in OXO-M (±MA pretreatment)-induced hypothermia. ANOVA applied specifically to data derived from rats receiving MA prior to OXO-M produced results that reflected those described above for rats receiving MA alone. There was no significant effect of age on the core body temperatures exhibited by rats in the MA + OXO-M treatment group and, FSL rats generally exhibited a greater reduction in core body temperature than the FRL rats, yielding a significant line difference for sensitivity to MA + OXO-M [line, $F(1, 162) = 17.73$, $p < 0.001$].

Analysis of the data from rats given SAL prior to OXO-M revealed both a significant main effect of age, $F(7, 179) = 89.70$, $p < 0.001$, and line, $F(1, 179) = 7.11$, $p < 0.01$, where FSL rats generally displayed a greater decrease in core body temperature after OXO-M (Fig. 1a). Figure 1(a) highlights the diminishing sensitivity of both FSL and FRL rats to the hypothermic effect of OXO-M from P10 to P21 [age, P10–P21, $F(4, 112) = 45.96$, $p < 0.001$]. There was also a significant line difference across this age range, where FSL rats were more sensitive to the hypothermic effect of OXO-M than the FRL rats [line, P10–P21, $F(1, 112) = 4.81$, $p < 0.05$]. At P21, there was a clear attenuation of OXO-M's ability to induce hypothermia in both lines of rat, and through P50, there was no significant effect of age.

Figure 1b shows age-dependent changes in OXO (±MA pretreatment)-induced hypothermia. Analysis of the MA + OXO group showed significant age, $F(7, 175) = 8.26$, $p < 0.001$, and line, $F(1, 175) = 59.86$, $p < 0.001$, effects, where the sensitivity of both lines to MA + OXO-induced hypothermia increased with age and FSL rats were more sensitive to the hypothermic effect than the FRL rats. From P10 to P18 rats given MA prior to OXO followed the same profile as that observed for the MA and MA + OXO-M groups described

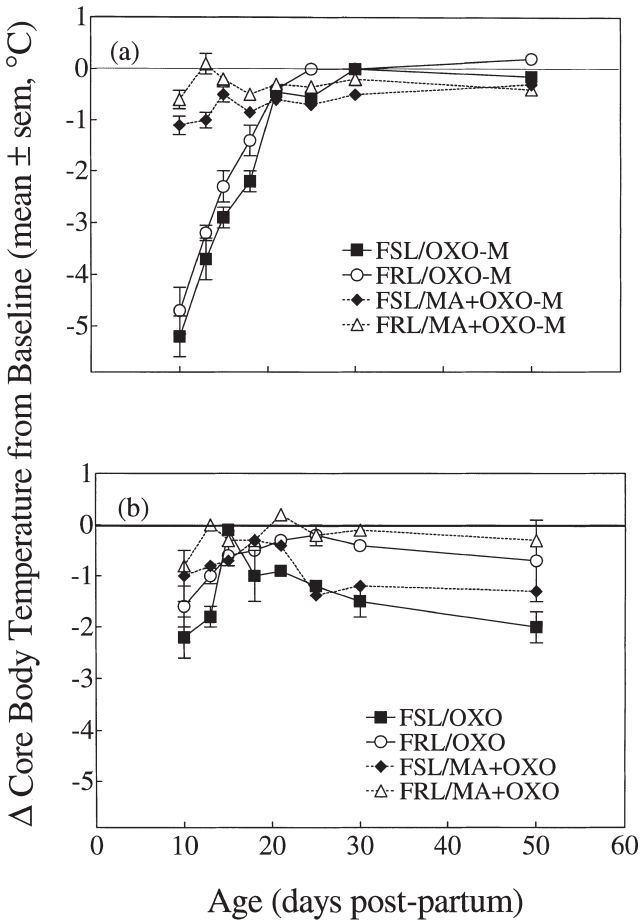


FIG. 1. Age-dependent changes in core body temperature after (a) OXO-M (±MA) and (b) OXO (±MA). There were no significant gender differences to P50 inclusive, so data for male and female rats were pooled. Each point represents the mean ± SEM from 8–16 rats for FSL and 7–14 for FRL rats. Each animal served as its own control, and change in core body temperature is with respect to baseline core body temperature. Error bars, which are not visible, are smaller than the symbol.

above. However, after P18, rats pretreated with MA followed a similar developmental profile to rats receiving SAL prior to OXO (vide infra). After P18, FSL rats were consistently more sensitive to MA + OXO-induced hypothermia than their FRL counterpart [line, $F(1, 93) = 109.98$, $p < 0.001$] and sensitivity to the hypothermic effect of MA + OXO increased with age [age, P21–P50, $F(3, 93) = 3.84$, $p < 0.01$]. An interaction effect between line and age was observed where FSL, relative to FRL rats, became progressively more sensitive to MA + OXO-induced hypothermia [line × age, $F(3, 93) = 3.78$, $p < 0.001$].

As depicted in Fig. 1b OXO-induced hypothermia, with no MA pretreatment, also varied significantly with age [age, $F(7, 170) = 13.10$, $p < 0.001$] and line [line, $F(1, 170) = 37.15$, $p < 0.001$], and did so in a line-dependent manner [line × age, $F(7, 170) = 5.22$, $p < 0.001$]. Up to and including P18, sensitivity of both lines to OXO diminished with age [age, P10–P18, $F(3, 77) = 14.26$, $p < 0.001$], and subsequently increased [P21–P50, $F(3, 93) = 7.27$, $p < 0.001$]. It should be noted that prior to P18 the lines were only significantly different in their response to OXO at P13, $t(14) = 1.76$, $p < 0.05$. After P18, the FSL rats were always more sensitive to the hypothermic effect of OXO [P21–P50, $F(1, 93) = 82.55$, $p < 0.001$]. The magnitude of the sensitivity difference between the lines increased with age, producing a significant interaction effect between line and age [P21–P50, $F(3, 93) = 3.43$, $p < 0.05$].

Dose–response studies: (a) oxotremorine-M. Figure 2 illustrates the dose–response relationship for OXO-M–induced hypothermia in 18- and 100-day-old FSL and FRL rats. There were no significant gender differences, so the results represent pooled male and female data. Inspection of the curves for 18 day olds, suggests that maximal hypothermia was not attained. However, due to the profound peripheral effects of OXO-M (e.g., lacrimation, salivation, and diarrhea), which were observed at the highest dose used here, increasing the dose further was deemed impractical. For the purpose of fitting the curves to the data, the hypothermia produced at the highest dose (0.6 μmol/kg) was entered as the E_{max}. Typical dose–response curves were obtained for both lines, but the curve derived for the FSL rats was shifted to the left of that for the FRL rats. The computer-derived ED_{50}s were 0.26 μmol/kg and 0.37 μmol/kg for FSL and FRL rats, respectively, and a Student's *t*-test, calculated using the computer SEM estimate, showed these to be significantly different, $t(16) = 13.79$, $p < 0.01$.

Data from P100 rats could not be fitted to the four parameter logistic equation for a sigmoid curve, so they are represented in Fig. 2 by a simple point-to-point line. The only significant effect of OXO-M was in FSL rats and at the highest dose, where a mean decrease in core body temperature of 0.8°C from baseline was observed, $t(8) = 8.0$, $p < 0.001$. A significant line difference was apparent at the highest dose (0.6 μmol/kg), where FSL rats showed a hypothermic response, and the core body temperature of FRL rats did not differ from baseline.

(b) Oxotremorine. Figure 3a illustrates dose–response curves for OXO-induced hypothermia in 13-day-old FSL and FRL rats. Again, gender differences were not significant, so the

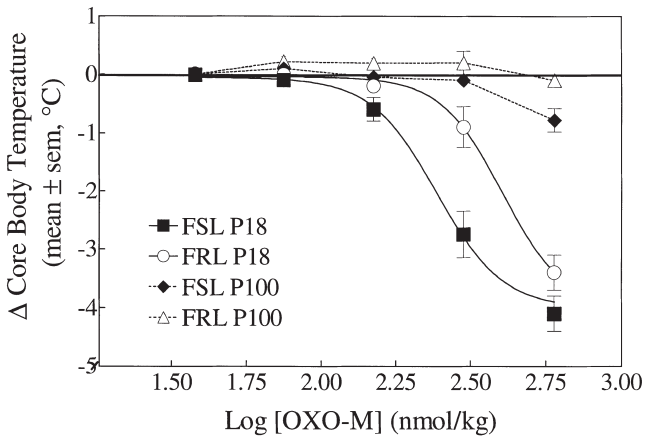
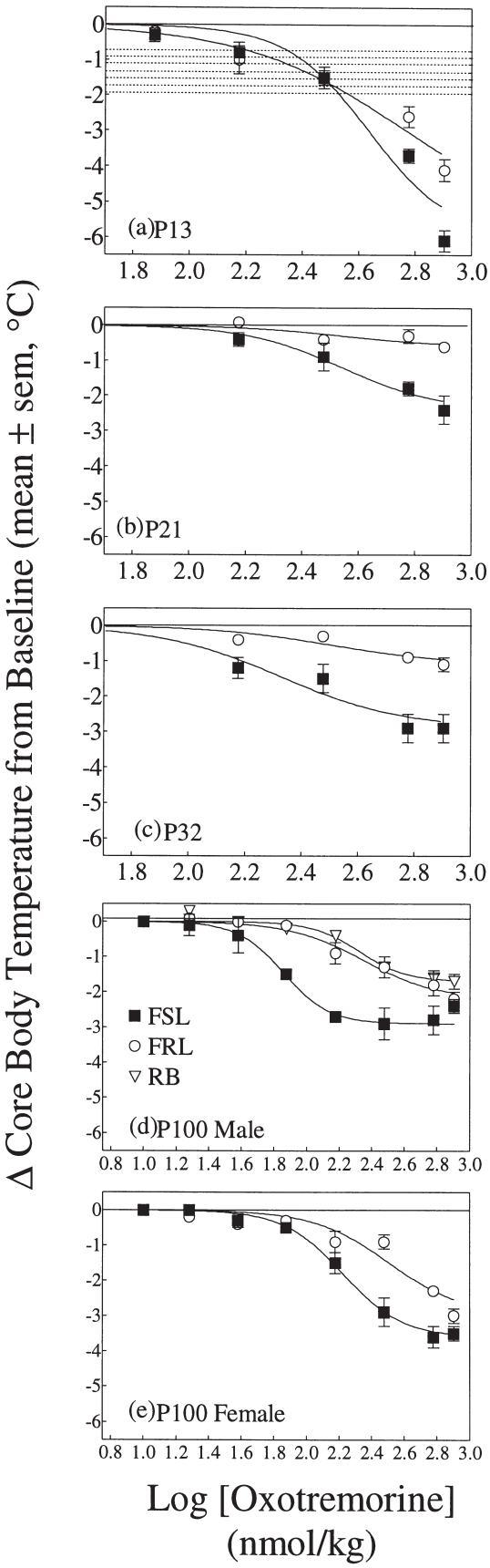


FIG. 2. Dose–response curves for OXO-M–induced hypothermia for P18 and P100 FSL and FRL rats. There were no significant gender differences, so data for males and females were pooled. At P18 each point represents the mean ± SEM from 5–10 FSL rats and 11–12 FRL rats and at P100, both FSL and FRL were represented by 5–9 rats per dose. Each animal served as its own control, and change in core body temperature is with respect to baseline core body temperature. Error bars, which are not visible, are smaller than the symbol.



data for males and females were pooled. FSL and FRL rats given MA pretreatment did not differ in their hypothermic response to OXO. Furthermore, a dose–response relationship was not evident. Hence, for the sake of clarity, these data have been represented on Fig. 3a as a stippled band spanning the maximal and minimal range of MA + OXO-induced hypothermia observed for both rat lines.

Superimposed on the "MA + OXO band" are the results from rats given OXO without MA pretreatment. In contrast to rats pretreated with MA, a dose–response relationship was apparent. A maximal hypothermia did not appear to be attained for either rat line, so the values obtained from rats given the highest dose of OXO (0.8 μmol/kg) were entered as the E_{max} values for computer fitting of the data. ED_{50}s were 0.47 μmol/kg and 0.34 μmol/kg for FSL and FRL rats, respectively, but were not significantly different.

With the exception of rats aged P13 (vide supra), dose–response curves were compiled using rats pretreated with MA to assess the central effects of OXO. Although SAL did not produce any significant change in core body temperature, methyl atropine generally produced hypothermia; however, this was slight in rats older than P13. Thus, the data depicted in Fig. 3b–e represent change in core body temperature for rats given MA + OXO minus any change in core body temperature, albeit slight, observed in control MA-treated rats.

At P21 (Fig. 3b) the dose–response curves for both lines were shallow compared to those at other ages, i.e., the maximal hypothermia was not as great. FSL rats displayed a greater maximal hypothermia than FRL rats, $t(18) = 4.43$, $p < 0.001$. ED_{50}s were 0.45 μmol/kg and 0.44 μmol/kg for FSL and FRL rats, respectively, and were not significantly different.

At P32 (Fig. 3c) the dose–response for both lines was shifted to left of that for rats aged P21, and there was an increase in the maximal hypothermia observed. Again, the FSL rats showed a greater maximal response compared to the FRL, $t(19) = 4.54$, $p < 0.001$. Furthermore, the FSL rats exhibited an ED_{50} of 0.23 μmol/kg that was significantly lower than that of 0.39 μmol/kg for the FRL rats, $t(19) = 5.09$, $p < 0.001$.

In P100 rats (Fig. 3d–e) there was a further leftward shift of the dose–response curve and a further increase in the magnitude of the maximal hypothermia. The FSL rats displayed a significantly lower ED_{50} [males, 0.075 μmol/kg, $t(7) = 24.67$, $p < 0.001$; females, 0.18 μmol/kg, $t(10) = 12.04$, $p < 0.001$], than the FRL rats [males 0.23 μmol/kg, females 0.34 μg]. The line differences in maximal hypothermia were no longer significant. There were gender-dependent differences in OXO-induced hypothermia at P100. The ED_{50} for males was lower than that for females, irrespective of line [FSL, $t(6) = 29.6$, $p < 0.001$; FRL, $t(11) = 8.71$, $p < 0.001$]. Females tended to show a slightly greater maximal hypothermia than males, although

FIG. 3. Age-dependent changes in the dose–response curves for OXO-induced hypothermia. Each animal served as its own control, and change in temperature is with respect to baseline core body temperature. FSL rats are shown as filled squares and FRL rats as open circles. In d, randomly bred Sprague–Dawley rats are represented by open triangles. The sample sizes are indicated in brackets; (a) P13, FSL [4–7]; FRL [6–11], (b) P21 FSL [7–8]; FRL [7–12], (c) P32, FSL [7–9]; FRL [9–12], (d) P100 males, FSL [3–6]; FRL [5–10]; Random Bred (RB) , (e) P100 females, FSL [4–6]; FRL [6–10]. Because there were no significant (within-line) gender differences at P13, P21, and P32, data for male and females were pooled. Error bars, which are not visible, are smaller than the symbol.

this only reached significance for FRL rats, $t(11) = 2.81$, $p < 0.05$. Also illustrated in Fig. 3d is the dose–response curve for OXO-induced hypothermia in randomly bred male Sprague–Dawley rats. In agreement with earlier studies (42,44), randomly bred Sprague–Dawley rats did not differ from FRL rats in their hypothermic response to OXO, suggesting that selection pressure did not serve to generate a line that is resistant to OXO-induced hypothermia. However, more recent studies have indicated that both FSL and FRL rats have diverged from Sprague–Dawley rats (49). Therefore, the developmental profiles of the FSL and FRL rats to cholinergic agents might reflect both the relative sensitivity of the FSL rats and the relative resistance of the FRL rats. Alternatively, given the many substrains of randomly bred Sprague–Dawley rats that exist, it is possible that in the present study a substrain was used that resembled the FRL rats for this phenotype.

Development of Drug-Induced Changes in Locomotor Activity

Dose–response studies: (a) oxotremorine-M. Figure 4 shows the dose–response curve for OXO-M–induced changes in locomotor activity in 18- and 100-day-old FSL and FRL rats. No significant gender differences were established, so the data for males and females were pooled. At P18, low doses of OXO-M produced hyperactivity in FRL rats, whereas FSL rats did not show any significant change from baseline. At the highest dose, locomotor activity decreased to approximately 30% of baseline levels for both FSL and FRL rats. The dose–response curve for the FSL rats was shifted to the left of that for the FRL. Computer-generated ED_{50}s were 0.09 μmol/kg and 0.29 μmol/kg for FSL and FRL rats, respectively, and these were significantly different, $t(160 = 7.17$, $p < 0.001$.

At P100 the locomotor activity was decreased to approximately 60% of baseline levels for both FSL and FRL rats. The dose–response curve for FSL moved to the right of that at P18, whereas the converse occurred for FRL. Computer-derived ED_{50}s were 0.18 and 0.24 μmol/kg for FSL and FRL rats, respectively, and were not significantly different. At the highest dose, although not quantified, lacrimation, salivation, piloerection, and diarrhea were observed in all rats.

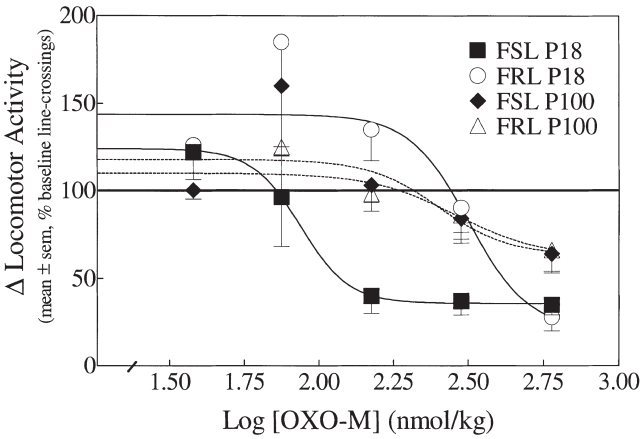


FIG. 4. Dose–response curves for OXO-M–induced changes in locomotor activity for P18 and P100, FSL and FRL rats. There were no significant gender differences, so data for males and females were pooled. At P18, each point represents the mean ± SEM from 5–10 FSL rats and 11–12 FRL rats, and at P100, both FSL and FRL were represented by five to nine rats per dose. Each animal served as its own control, and change in locomotor activity is expressed as a percent of baseline locomotor activity. Error bars, which are not visible, are smaller than the symbol.

(b) Oxotremorine. Table 1 lists the changes in locomotor activity observed in control rats receiving either SAL or MA. Generally, SAL and MA did not produce any significant change in locomotor activity from baseline, although there was a trend for hyperactivity in rats aged P21. This is in accordance with the developmental profile of baseline locomotor activity (17). Due to the high variability associated with the mean and the fact that SAL and MA-induced changes were generally within error of baseline scores, the results illustrated in Fig. 5a–d represent the percentage change from baseline activity after MA + OXO with no adjustment for SAL or MA-induced changes in locomotor activity.

There were no significant gender differences at any of the ages tested; thus, the following results represent pooled male and female data. Figure 5a illustrates dose–response curves for OXO (± MA)-induced changes in locomotor activity in 13-day-old rats. Typical dose–response curves were obtained for rats receiving either MA + OXO or OXO alone, and within each line the calculated ED_{50}s were not significantly different. The curves for FSL rats were shifted to the left of those for FRL rats. Computer-generated ED_{50}s for FSL rats were 0.24 and 0.27 μmol/kg for OXO and MA + OXO, respectively, and for the FRL rats, the ED_{50} was 0.48 μmol/kg for both OXO and MA + OXO dose–response curves. For both treatments the ED_{50} for FSL rats was significantly lower than that for FRL rats [MA + OXO, $t(12) = 3.64$, $p < 0.01$; OXO, $t(10) = 2.42$, $p < 0.05$].

At P21 (Fig. 5b) the dose–response curve for FRL rats was shallower than at P13, with maximal suppression of locomotor activity being around 50% of baseline. Likewise, the maximal MA + OXO-induced suppression of locomotor activity was not as great as at P13, being approximately 30% of baseline at P21. The difference in E_{max} between the lines was significant, $t(18) = 2.13$, $p < 0.05$. There was a small leftward shift in the dose–response curves for both lines (FSL = 0.21 μmol/kg; FRL = 0.42 μmol/kg). The dose–response for the FSL rats was unusual in that hyperactivity was observed after saline and at low doses of OXO. Thus, the ED_{50} dose produced no change in locomotor activity from baseline.

At P32 (Fig. 5c) the dose–response for both lines was shifted to the right of that for rats aged P21 and the ED_{50}s for FSL (0.26 μmol/kg) and FRL (0.46 μmol/kg) were significantly different, $t(19) = 19.36$, $p < 0.001$. The FRL rats showed a slight increase in maximal suppression of locomotor activity (30% of baseline) compared to P21, whereas the E_{max}

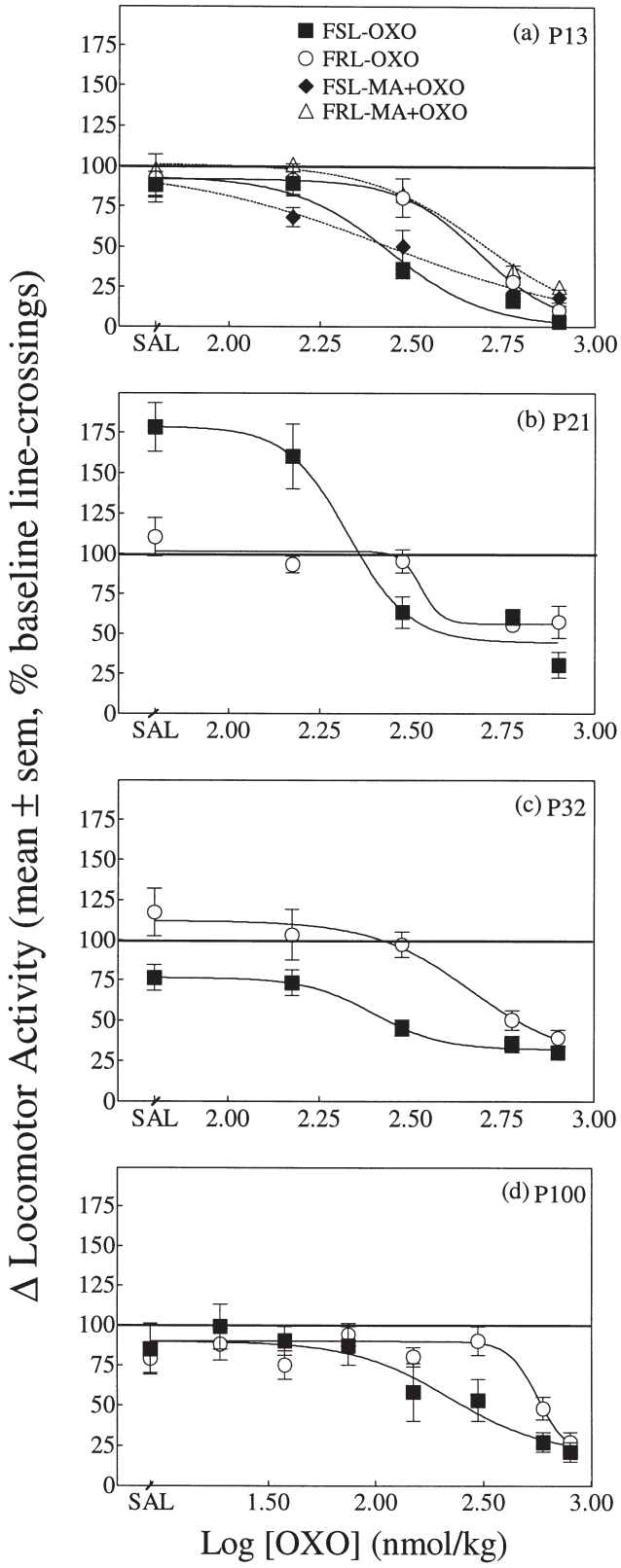
TABLE 1

AGE-DEPENDENT CHANGES IN LOCOMOTOR ACTIVITY IN CONTROL FSL AND FRL RATS

	Saline		Methyl Atropine*	
Age	FSL	FRL	FSL	FRL
P13	88 ± 18†	90 ± 12	92 ± 33	133 ± 33
P21	179 ± 17	114 ± 13	125 ± 14	132 ± 16
P32	77 ± 8	117 ± 23	84 ± 19	106 ± 14
P100	85 ± 17	79 ± 8	81 ± 11	105 ± 16

*Rats were given SAL or MA (0.73 mmol/kg).

†Values are expressed as a percentage of baseline and are expressed as mean ± SEM. There were 4–20 rats per group.



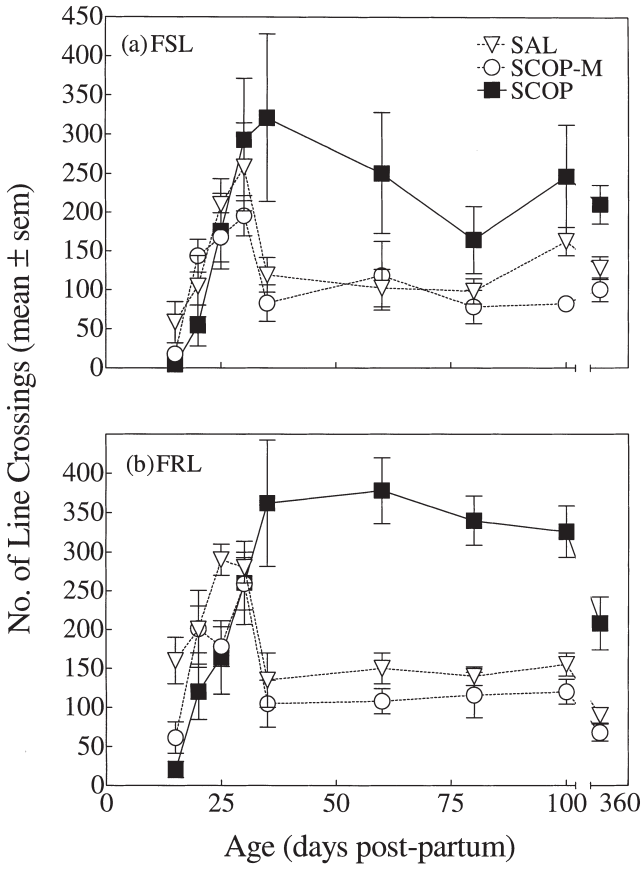


FIG. 6. Age-dependent effect of SAL, SCOP-M, and SCOP on locomotor activity in (a) FSL and (b) FRL rats. Each point represents the mean ± SEM from 5–10 male rats. Line-crossings were over a 5-min test.

for FSL rats was not different. In P100 rats (Fig. 5d) there was a further rightward shift of the dose–response curve for both lines. The ED_{50} for the FSL rats (0.28 μmol/kg) was significantly lower than that for the FRL rats (0.65 μmol/kg), $t(7) = 14.09$, $p < 0.001$. Again, there was no significant difference in the Emax of FSL and FRL rats.

Development of sensitivity to scopolamine. Figure 6a and b illustrates age-dependent changes in the absolute number of line-crossings made in a 5-min test for each of the three treatments (SAL, SCOP-M, and SCOP). Figure a and b represents the results for FSL and FRL males, respectively. Three-way ANOVA revealed significant main effects of age, $F(8, 341) = 31.27$, $p < 0.001$, treatment, $F(2, 341) = 14.10$, $p < 0.001$, and

FIG. 5. Age-dependent changes in the dose–response curves for OXO-induced changes in locomotor activity. Each animal served as its own control and change in locomotor activity is expressed as a percentage of baseline locomotor activity. FSL rats and FRL rats are depicted by filled and open symbols respectively. The sample sizes are indicated in brackets; (a) P13, FSL [4–7]; FRL [6–11], (b) P21, FSL [7–8]; FRL [7–12], (c) P32, FSL [7–9]; FRL [9–12], (d) P100 FSL [7–12]; FRL [11–20]. Because there were no significant (within-line) gender differences at any of the ages tested, data for male and females were pooled throughout. Error bars, which are not visible, are smaller than the symbol.

line, $F(1, 341) = 8.98$, $p < 0.01$, with a significant interaction between age and treatment, $F(16, 341) = 6.50$, $p < 0.001$.

Analyses performed on each treatment group individually revealed significant age-dependent changes in locomotor activity for all treatments [SAL, $F(8, 117) = 25.84$, $p < 0.001$; SCOP-M, $F(8, 111) = 17.45$, $p < 0.001$; SCOP, $F(8, 111) = 10.46$, $p < 0.001$]. There were no significant line or line by age interactions. Figure 6a and b shows a peak in activity from P25 to P30 in FSL and FRL rats for all treatments but, whereas SAL or SCOP-M–treated rats showed a subsequent reduction in activity, the SCOP-treated rats remained hyperactive. Thus, the significant main effect of treatment, and age by treatment interaction, detected by the three-way ANOVA, was attributable to the SCOP treated rats (Fig. 6a and b) which, after P30, were hyperactive relative to SAL and SCOP-M control groups [main effect, treatment, P30–P360, (a) FSL, SAL/SCOP, $F(4, 54) = 6.05$, $p < 0.001$; SCOP-M/SCOP, $F(4, 52) = 4.46$, $p < 0.004$, (b) FRL, SAL/SCOP, $F(4, 77) = 11.91$, $p < 0.001$; SCOP-M/SCOP, $F(4, 75) = 10.03$, $p < 0.001$]. Prior to this, the locomotor activity observed in SCOP-treated rats was lower or not different from the control rats. After P35, FRL rats were generally more hyperactive than the FSL rats after SCOP, although this did not reach significance due to the high variability associated with the mean. When the data were expressed as a percent of saline controls, nonparametric analyses showed significant line differences after P60, where FRL rats were more active after SCOP than the FSL rats, $U = 0$, $p < 0.05$.

Post hoc experiments using a lower dose of SCOP also provided support for a sensitivity difference between FSL and FRL rats aged P80. After SCOP, FSL rats exhibited line-crossing activity at 189 ± 16% of baseline compared to 251 ± 21% in FRL rats. Here, each animal served as its own control, and so parametric statistical analyses were performed on the absolute data. The difference in activity between the lines was significant, $t(43) = 2.20$, $p < 0.05$. These values were not significantly different from those derived at the higher doses of SCOP, providing no evidence for a dose–response relationship within this range.

Ontogeny of binding sites for [^{3}H]-QNB: (a) cerebral cortex. In initial experiments the ontogeny of mAChR in the cerebral cortex was investigated. The data illustrated in Fig. 7 were for pooled male and female samples to yield mean values (n = 6–8 per group). The rationale for this was validated by the lack of difference between the sexes for binding to cortical mAChR. B_{max} estimates from [^{3}H]-QNB (0.7 nM) single-point binding were calculated using K_d values determined from independent saturation binding assays. There was a significant increase in cortical mAChR with age, $F(2, 40) = 11.21$, $p < 0.001$, and an interaction effect between age and line, $F(2, 40) = 7.53$, $p < 0.01$. FSL rats possessed significantly fewer cortical mAChR at P19, $t(14) = 2.77$, $p < 0.01$, more at P31, $t(12) = 3.10$, $p < 0.01$, and similar numbers at P61.

(b) Striatum and hippocampus. Age-dependent changes in the Kd and B_{max} of [^{3}H]-QNB binding were determined from saturation assays. At P13 and P21, Kd values for the striatum (range = 0.07–0.10 nM) and hippocampus (range = 0.10–0.11 nM) were similar in FSL and FRL rats, with the largest variation being less than 10% at each age. Likewise, at P32 and P120 there was no apparent difference in the affinity of the mAChR for [^{3}H]-QNB between the two rat lines. In the striatum, the K_d was similar to that observed in younger rats (range = 0.075–0.078 nM), while in the hippocampus there was an approximate twofold increase in the affinity of the mAChR for [^{3}H]-QNB (range = 0.055–0.069 nM). Scatchard

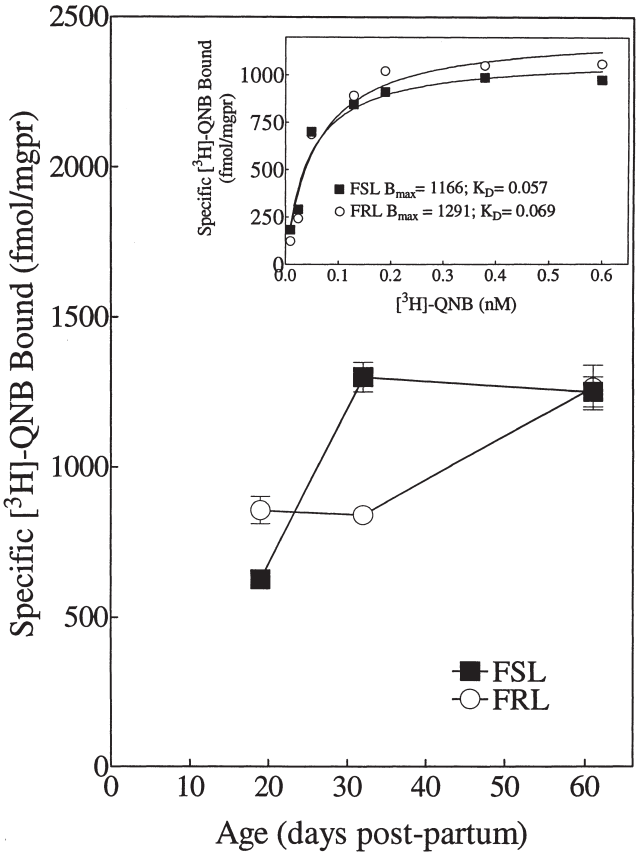


FIG. 7. Developmental changes in the binding capacity of [^{3}H]-QNB in the cerebral cortex of FSL and FRL rats. Estimated B_{max} values are expressed as the mean ± SEM (n = 6–8 per group). Inset: specific [^{3}H]-QNB saturation binding isotherms for adult (P100) male FSL and FRL cortical homogenates. Each point represents the mean of duplicate assays. Error bars, which are not visible, are smaller than the symbol.

analysis revealed no difference in B_{max} between the lines at P13 and P21 (Table 2). However, at P32, FSL rats displayed approximately 30 and 20% more mAChR in the striatum and hippocampus, respectively, than the FRL. Similarly, at P120 the FSL exhibited 27 and 24% more mAChR in the striatum and hippocampus (Table 2).

To validate B_{max} results derived from saturation analysis, binding was performed at a single, saturating, concentration of [^{3}H]-QNB, with a large enough sample size to allow statistical analyses. Male rats were used to avoid any confounding variability introduced through fluctuation of mAChR number during estrous in sexually mature female rats [e.g., (21,52)]. In agreement with saturation binding results, Fig. 8 shows that mAChR numbers differ between FSL and FRL rats at certain ages. For the striatum and hippocampus respectively, ANOVA revealed a significant main effect of age, $F(5, 66) = 12.42$, $p < 0.001$; $F(5, 55) = 114.75$, $p < 0.001$, line, $F(1, 66) = 21.85$, $p < 0.001$; $F(1, 55)$ 19.55, $p < 0.001$, and age by line interaction, $F(5, 66) = 3.72$, $p < 0.01$; $F(5, 55) = 12.06$, $p < 0.001$. The elevation in receptor number displayed by the FSL rats did not emerge until P32, and was in the order of 17 and 32% for the striatum and hippocampus, respectively. At P61, FSL rats possessed 33% more mAChR in both the striatum and hippocampus, and at P120, 25 and 24%, respectively. FSL

TABLE 2

THE ONTOGENY OF MAXIMAL mAChR NUMBER IN THREE BRAIN REGIONS OF THE FSL AND FRL RATS*

	Striatum		Hippocampus		Hypothalamus	
Age	FSL	FRL	FSL	FRL	FSL	FRL
13	737 ± 35	801 ± 68	274 ± 18	315 ± 47	248	233
21	1160 ± 155	1152 ± 136	726 ± 47	742 ± 33	463	426
32	1545 ± 135	1083 ± 128	1031 ± 37	800 ± 13	528	473
120	1897 ± 133	1378 ± 145	1257 ± 51	957 ± 23	429	357

*B_{max} values were determined from [^{3}H]-QNB saturation binding assays. Tissue samples for the striatum and hippocampus were pooled homogenates from three rats and the values below represent the means ± SEM of two replicate assays. Hypothalamic tissue was pooled from six rats and the results presented are from a single saturation assay. All values are expressed as fmol/mg protein.

rats showed higher levels of mAChR and a greater increase in mAChR with age.

(c) Hypothalamus. Saturation binding for the hypothalamus was performed in singlet because of the limited availability of tissue. Homogenates from six rats were pooled to provide sufficient tissue for equilibrium binding conditions to be met as well as PZ competition binding. These data revealed no line difference in the affinity of [^{3}H]-QNB for hypothalamic mAChR at any of the ages tested (range = 0.06–0.08 nM). FSL rats appeared to have marginally more mAChR at all ages with the difference being greatest (ca. 20%), at P120 (Table 2).

As illustrated in Fig. 8, single-point binding revealed no marked difference in maximal binding between the lines. There was a significant age effect, $F(5, 66) = 28.69$, $p < 0.001$, and an age by line interaction, $F(5, 66) = 2.85$, $p < 0.05$. Compared to the FRL, FSL rats exhibited greater maximal binding in the order of 6% at P13 to 17% at P120.

Ontogeny of M1 and M2 receptor subtypes. Inhibition of specific [^{3}H]-QNB binding with PZ was used to determine the ontogeny of the relative proportions of M1 and M2 receptor subtypes. Due to the lack of difference in the ratio of M1:M2 receptors over age, the results presented here focus on data derived from rats aged P13 and P120.

(a) Striatum. Results for inhibition of specific [^{3}H]-QNB binding, using PZ as the unlabeled competitor, generally did not reveal any striking difference in the ratio of M1:M2 receptor subtypes between the FSL and FRL rats. However, as Fig. 9 shows, there was some indication that ontogenetic deviations in the ratio of M1:M2 receptor subtypes occurred in striatal preparations derived from FSL rats. Competition curves depicted in Fig. 9 were fitted to a two-site model. In all cases the two-site fit was significantly better than the one-site, $F(2, 15)$ values ranged from 7.24 to 14.0, $p < 0.01$. The capacity of PZ to inhibit [^{3}H]-QNB binding in FRL rats was not significantly different between the ages of P13 and P100, indicating that the ratio of M1:M2 receptor does not change, at least after P13. In contrast, the FSL rats show an increase in M1 receptors from P13, where M1 receptors represent 65% of mAChR, to P120 where they account for 80%. This change appeared to be progressive with M1 accounting for 61% of mAChR at P21 and 70% at P32. Thus, the ratio of M1:M2 changes from approximately 2:1 at P13 to 4:1 by P120 in FSL rats, whereas for the FRL the ratio remains relatively constant at 2:1 during this developmental period. The ontogenetic change in the ratio of M1:M2 in FSL rats is represented in parts a and b of Fig. 9. Figure 9a shows no difference in PZ competition between FSL and FRL rats at P13, whereas by P120 the FSL rats exhibit a greater number of M1 receptors as seen by the shift to the right of the competition curve (Fig. 9b).

*K*i values ranged from 2.8 to 5.2 nM for the M1 high-affinity site, and 167 to 253 nM for the M2 low-affinity site, and showed no consistent change over the developmental ages examined. Furthermore, there was no apparent difference in *K*i values between the FSL and FRL rats. Of course, it is difficult to comment on the significance of these results because they are based on only two replicate assays at each age. Suffice it to say that, qualitatively, there is no difference in the affinity states of the M1 and M2 receptors between line or with age, but there appears to be an increase in the number of striatal M1 receptors exhibited by FSL rats during the development of the muscarinic cholinergic neurotransmitter system.

(b) Hippocampus. For the hippocampus, there was no marked difference in the ratio of M1:M2 receptors between FSL and FRL rats at any of the ages examined. The data fitted a two-site model significantly better than a one-site, indicating the presence of both high (M1)- and low (M2)-affinity receptors, $F(2, 15)$ ranging from 8.4 to 38.2, $p < 0.01$ across all lines and ages. For both lines the number of M1 receptors increased between P13 and P21, accounting for 45% of total mAChR at P13, 60% at P21 and P32 and 61%, and 65% at P120 for FRL and FSL, respectively. *K*i values were similar across the ages and between the lines, ranging from 2 to 5 nM for the M1 and 173 to 362 nM for the M2 receptor.

(c) Hypothalamus. PZ competition binding data in the hypothalamus was pooled for all ages. Pooling the data was justified by the lack of difference in PZ competition curves between the ages tested. A one-site model was fitted to the data, as the two-site model was not statistically valid. There was no marked differences between the FSL and FRL rats. For both lines the receptors were of low affinity (*K*i = 187.3 and 223.8 nM for FSL and FRL, respectively), indicating the presence of predominantly M2 receptors.

DISCUSSION

The main aim of this study was to examine the ontogeny of muscarinic sensitivity in the FSL rat, a model of human depression that was selectively bred for increased cholinergic function. It is now well recognized that humans with depressive disorders are more sensitive to the behavioral and physiological

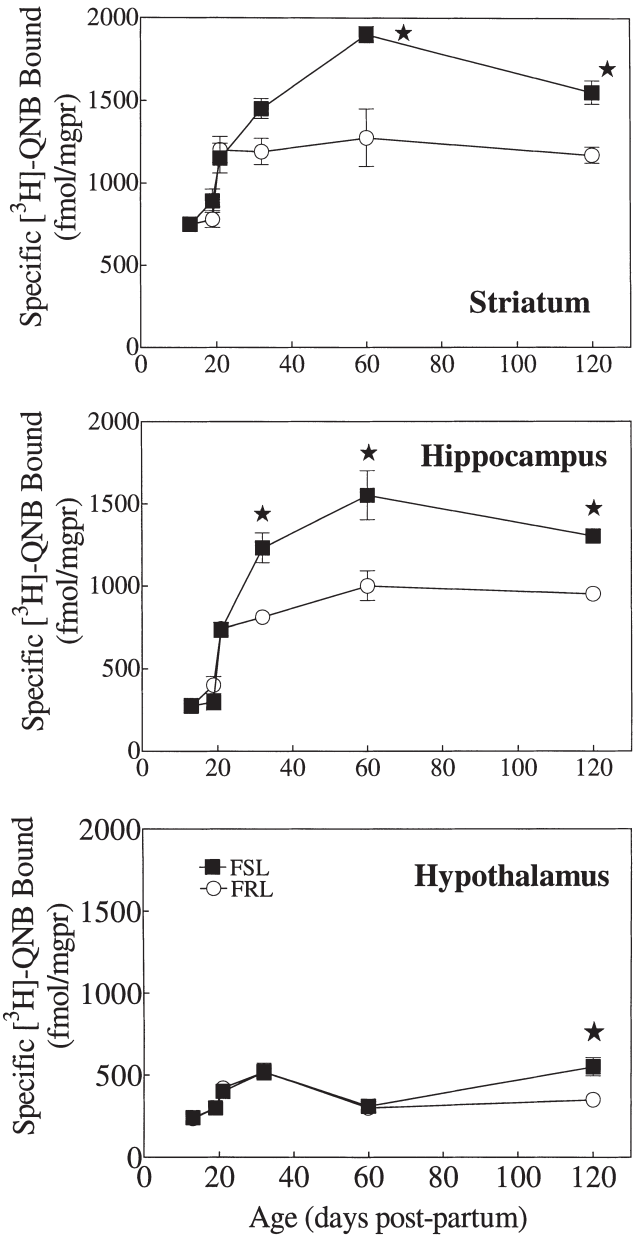


FIG. 8. Developmental changes in the binding capacity of [^{3}H]-QNB in the striatum, hippocampus, and hypothalamus of FSL and FRL rats. Estimated *B*max values are expressed as the mean ± SEM (n = 6–8 per group). FSL, filled squares; FRL open circles. FSL significantly differed from their FRL counterpart, $*p < 0.05$ *t*-tests. Error bars, which are not visible, are smaller than the symbol.

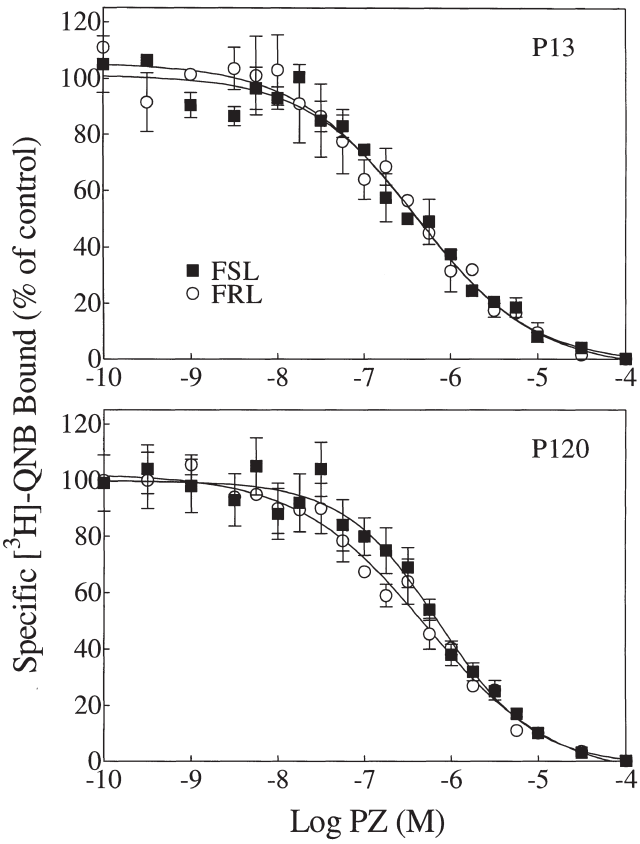


FIG. 9. PZ inhibition of [^{3}H]-QNB binding in the striatum of 13- and 120-day-old FSL and FRL rats. Data are the mean ± SEM of two replicate assays. [^{3}H]-QNB binding is expressed as the percent of binding in the absence of PZ. Error bars, which are not visible, are smaller than the symbol.

effects of muscarinic drugs than are nondepressed controls (6,25,55,62). Controversy remains as to whether this altered cholinergic function is a cause or a consequence of the depressive disorder (i.e., a trait or a state marker for the illness).

Development of Sensitivity to Muscarinic Agonists

This study represents, to the best of our knowledge, the first systematic behavioral and physiological study of the development of sensitivity to a muscarinic agonist (OXO) in rats. Other studies [e.g., (12)] investigating the development of behavioral sensitivity to agonists, for example, OXO, have used mice aged P21 or older. Thus, the data presented here provide information that may be heuristic in developing new hypotheses and a greater understanding of depressive disorders.

Data from both single-dose and dose–response agonist studies provide support for the hypothesis that increased muscarinic sensitivity is a trait marker for the "depressive" characteristics of the FSL rats. The FSL rats were more sensitive to the hypothermic and locomotor suppressant effects of OXO and OXO-M early postnatally, and this was reflected by ontogenetic shifts in both the ED_{50} and E_{max}.

An interesting feature of the development of sensitivity to the hypothermic and locomotor suppressant effects of OXO is the "inverted U-shape" function for age produced by a period of relative insensitivity to OXO during the second to third postnatal week. This was particularly apparent when comparing *E*max values across the postnatal ages tested. To the best of our knowledge, the literature describing the development of behavioral sensitivity to muscarinic antagonists does not follow such a pattern. For example, there appears to be a developmental window for the emergence of SCOP (antagonist)-induced hyperactivity at P25–P30, and a paradoxical hypoactivity prior to this [(2,7,10); present study]. In contrast, OXO-induced hypoactivity was greatest as early as P13 and again at adulthood.

The increased physiological and behavioral sensitivity of FSL and FRL rats to muscarinic agonists early postnatally parallels other reports of increased responsiveness of the muscarinic system during development. For example, muscarinic agonist-induced increases in phosphoinositide hydrolysis are much greater in 7- and 14-day-old rats than in 21-day-old rats (3,25,56). De Vries and co-workers (20) reported that the first measurable effects of OXO activation of mAChR on the electrically evoked release of [^{3}H]-acetylcholine (ACh) appeared at P7, where ACh release was greatly inhibited. However, over the following 2 weeks the inhibitory effect of OXO decreased. Zahalka and co-workers (68) reported high levels of [^{3}H]hemicholinium-3 binding, a specific marker for the choline transport site, late in gestation and immediately after birth. In particular, there was a minor peak in binding at P10 in the cerebral cortex that corresponded to a transient period of cholinergic hyperactivity in this brain region. They proposed that cholinergic tone may be high during a period when there are relatively few cholinergic nerve terminals or alternatively, binding sites may be transiently expressed by noncholinergic cells. Thus, the pattern of sensitivity to muscarinic agonists, as indexed by OXO-induced hypothermia and suppression of locomotor activity, may be a reflection of such ontogenetic changes in cholinergic tone where FSL rats, relative to the FRL, exhibit cholinergic hyperactivity.

With respect to measures of core body temperature, it is unlikely that an inability of rat pups aged P10–P13 to thermoregulate can fully account for the marked hypothermia observed after OXO. This age is consistent with that for the acquisition of thermoregulatory abilities of around P11–P12 in other strains of rats (15,67). Moreover, the decrease in core body temperature exhibited by the rat pups after SAL was slight relative to that observed after OXO or OXO-M and indicates that, at least under the present conditions, rat pups were able to thermoregulate adequately.

Correlations With the Development of the BBB

At postnatal ages prior to P21, effects of OXO-M on central muscarinic receptors cannot be overlooked. Perhaps the most striking result was that of the substantial hypothermia and suppression of locomotor activity produced by OXO-M in rat pups younger than P21. OXO-M is more potent than its tertiary analog, OXO, but does not readily cross the BBB in adult rats (53). Interestingly, rats aged P13 which were given MA prior to OXO did not exhibit hypothermia, suggesting that MA was blocking the receptors mediating this response. Although the present data do not provide a direct measure of BBB integrity, the postnatal age (P21) when OXO-M ceased to exert a hypothermic effect and the MA ceased to block the effects of OXO, corresponds well with the reported time frame for BBB maturation in rats (54,61,64), and thus, it is likely that these results reflect such.

Dose–response curves for OXO (±MA) at P13 and for OXO-M at P18 provide further support for BBB permeability to these drugs early postnatally. Both OXO and OXO-M produced dose-dependent decreases in core body temperature and locomotor activity. However, rats aged P13 and pretreated with MA did not exhibit OXO-induced hypothermia suggesting that MA was blocking the receptors mediating this response. By contrast, OXO-induced hypoactivity was apparent in the presence or absence of MA, implying that this response was centrally mediated and that the receptors involved were protected from MA by the BBB. This supports the findings of others that various components of the BBB develop at different rates [e.g., (9,61)].

Development of Sensitivity to Muscarinic Antagonists

For both FSL and FRL rats, SCOP-induced hyperactivity emerged during the fifth postnatal week and was preceded by a paradoxical hypoactivity. This conforms to previous reports indicating that there is a developmental period for the emergence of SCOP-induced hyperactivity at P25–P30 (2,7,10). Moreover, saline and SCOP-M–treated control rats exhibited activity profiles that agree with earlier studies describing a period of hyperactivity during the second to third postnatal week followed by a decrease to "adult" locomotor activity levels [e.g., (7,8,11,39)]. That locomotor activity in FSL and FRL rats develops in parallel with that observed in other rat strains suggests that the differences in activity between FSL and FRL rats after SCOP is a consequence of altered sensitivity to the drug and not a confound of the FSL and FRL rats differing in their state of behavioral arousal during development.

In contrast to agonist studies, where FSL rats exhibited muscarinic supersensitivity at the earliest age tested, FSL rats did not display subsensitivity to the muscarinic cholinergic antagonist, SCOP, until P60. Thus, it appears that sensitivity to muscarinic agonists appears earlier than that for antagonists. Perhaps the most parsimonious explanation for the earlier emergence of sensitivity to muscarinic agonists than antagonists lies in their basic pharmacology. Specifically, agonists possess the properties of affinity (as do antagonists) and also efficacy. It could be argued that an agonist can have an effect as soon as receptors are present, whereas an antagonist may require further integration of the cholinergic system with other systems. This is analogous to studies that have reported that scopolamine is unable to potentiate amphetamine-induced hyperactivity until the cholinergic and adrenergic systems have become integrated (10). It has been suggested that during the third postnatal week rats are already hyperactive because of the earlier maturation of the adrenergic system. Scopolamine cannot stimulate prior to that age because the two systems are not yet integrated (10,11). It appears, then, that the appearance of altered muscarinic sensitivity in FSL rats is different for agonists and antagonists, and may be correlated with muscarinic receptor number and/or affinity and/or integration of the cholinergic system with other systems.

Ontogeny of Muscarinic Receptors

In both FSL and FRL rats, mAChR number increased with age in the cortex, hippocampus, and striatum, to be at, or near, adult levels by the end of the fourth postnatal week. In the hypothalamus, mAChR number remained relatively constant, although there was a small increase from P13, the earliest age tested, to P32. These results are in good agreement with other reports (4,16,33,65). Competition binding studies were also in good agreement with other studies where M1 binding sites have been reported to increase from 30% of adult levels at P7 to 100% by P28 (1,35), and where M2 receptors show little postnatal change (35) or a gradual increase to reach adult levels around the end of the first postnatal month (1,33,34,38).

As previously reported by Overstreet and co-workers (44,51), there was no difference in mAChR density in the mature cortex of FSL and FRL rats. By P32, FSL rats possessed significantly more hippocampal and striatal [^{3}H]-QNB binding sites. In the hippocampus, the ratio of M1:M2 receptors between the FSL and FRL rats was the same, while in the striatum, FSL rats appeared to have marginally more M1 receptors relative to FRL rats. In the hypothalamus, there was no difference in receptor number until well into adulthood. Thus, the present data provide no evidence for mAChR number and/or affinity per se being primary in mediating the differ-

ence in behavioral and physiological sensitivity between FSL and FRL rats observed after treatment with muscarinic agonists early postnatally. This finding agrees with other reports of "mismatches" between functional responses and receptor change. For example, Collins et al. (14) found that the number of [^{3}H]-QNB binding sites did not correlate with the differing sensitivity of two strains of mice to OXO. The present data suggest that systems downstream from the mAChRs may better account for the behavioral sensitivity differences to muscarinic agonists between the FSL and FRL rats than receptor number per se, for example, coupling of mAChR to its second messengers. This is an intriguing hypothesis, given recent interest in the possibility of alterations in g-protein function in human depressives (41,50), and the fact that FSL rats are also more sensitive to the hypothermic effects of a serotonin1A agonist, which is coupled to the same second messengers as the M2 subtype of the mAChR [e.g., (47)].

Implications for Human Depression

Clearly, the neurochemical basis(es) underlying the early postnatal development of sensitivity to muscarinic drugs cannot be answered from this study and remains open to speculation. Likewise, so does the mechanism underlying the muscarinic supersensitivity in the FSL rat. The present study does, however, provide a number of implications for human depressive disorders. First, as has been suggested previously (18,45), the lack of difference in the number of mAChRs in brain tissue derived from suicide victims [e.g., (32)] should not be considered evidence against cholinergic involvement in depression. It is clear from the present study that there is no simple relationship between muscarinic sensitivity and mAChR number. In fact, there are direct parallels between the human and animal literature in that there has been a failure to show changes in mAChR number in the cortical preparations, while demonstrations of muscarinic sensitivity differences are quite easily observed after pharmacological manipulation (6,60). Moreover, that chronic treatment with an anticholinesterase known to downregulate muscarinic receptors did not have an antiimmobility effect in the FSL rats (59) questions the relationship between mAChRs and depression. Nevertheless, the present data are consistent with recent studies of supersensitive muscarinic cholinergic responses in children genetically at risk for developing a depressive disorder (60), providing support for muscarinic supersensitivity as a trait marker of depressive disorders. Future studies using the FSL rats should provide further insight into possible mechanisms underlying the etiology of depression and further probe the mechanism of muscarinic cholinergic hyperfunction.

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