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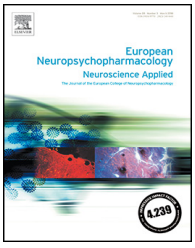

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Sex-dependent changes in ketamine-induced locomotor activity and ketamine pharmacokinetics in preweanling, adolescent, and adult rats

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Abstract
Although ketamine has long been known to increase locomotor activity, only recently was it realized that this behavioral effect varies according to both sex and age. The purpose of the present study was threefold: first, to measure the locomotor activating effects of ketamine in male and female rats across early ontogeny and into adulthood; second, to assess ketamine and norketamine pharmacokinetics in the dorsal striatum and hippocampus of the same age groups; and, third, to use curvilinear regression to determine the relationship between locomotor activity and dorsal striatal concentrations of ketamine and norketamine. A high dose of ketamine (80 mg/kg, i.p.) was administered in order to examine the complete cycle of locomotor responsiveness across a 280-min testing session. In separate groups of rats, the dorsal striata and hippocampi were removed at 10 time points (0–360 min) after ketamine administration and samples were assayed for ketamine, norketamine, and dopamine using HPLC. In female rats, ketamine produced high levels of locomotor activity that varied only slightly among age groups. Male preweanling rats responded like females, but adolescent and adult male rats exhibited lesser amounts of ketamine-induced locomotor activity. Ketamine and norketamine pharmacokinetics, especially peak values and area under the curve, generally mirrored age- and sex-dependent differences in locomotor activity. Among male rats and younger female rats, dorsal striatal ketamine and norketamine levels accounted for a large proportion of the

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variance in locomotor activity. In adult female rats, however, an additional factor, perhaps involving other ketamine and norketamine metabolites, was influencing locomotor activity.

1. Introduction

Ketamine is a NMDA receptor channel blocker that was approved by the Food and Drug Administration nearly five decades ago (1970) for use as a dissociative anesthetic (Domino, 2010; Kohrs and Durieux, 1998). Ketamine's unique properties make it an especially effective anesthetic for hypotensive patients and pediatric populations (Bergman, 1999; Morris et al., 2009). More recently, it was discovered that low-dose ketamine treatment quickly reduces symptoms associated with treatment-resistant depression (aan het Rot et al., 2010; Zarate et al., 2006). Although having clear medical utility, ketamine, either alone or in combination with other drugs (e.g., MDMA, amphetamine, or cocaine), is often used illicitly (Dillon et al., 2003; Jansen, 1993, 2000). The prevalence rate of ketamine abuse varies substantially world-wide, as some countries report almost no recreational use; whereas, ketamine is among the most frequently used illicit drugs in Hong Kong and some southeast Asian nations (Kalsi et al., 2011; Morgan and Curran, 2012; Tan et al., 2012). The overall prevalence rate of ketamine abuse in the United States is uncertain, but the rate of ketamine use among 12th graders was 1.5% (2014), which was greater than for either phencyclidine (PCP) or methamphetamine (Johnston et al., 2015). When used recreationally, ketamine is typically self-administered at high doses and shows rapid tolerance (Dillon et al., 2003; Jansen, 2000).

In addition to inducing anesthesia, one of the most striking behavioral characteristics of ketamine is its ability to stimulate locomotor activity (Usun et al., 2013; Yamamoto et al., 2016). Specifically, adult male rats and mice injected with moderate to high doses of ketamine (20–150 mg/kg) typically exhibit an initial period of hypoactivity, presumably due to the drug's anesthetic and ataxic actions, and then an extended period of hyperactivity (Hetzler and Wautlet, 1985; Irifune et al., 1991). More recent studies have shown that sex and age are important contributing factors that interact to modulate ketamine-induced locomotor activity (McDougall et al., 2017; Wilson et al., 2005, 2007; but see Zanos et al., 2016). Among male rats, ketamine stimulates progressively less locomotor activity as the age of the animal increases (McDougall et al., 2017; Rocha et al., 2017). In contrast, female rats exhibit high levels of ketamine-induced locomotion that remain stable across early ontogeny (McDougall et al., 2017). Thus, available evidence suggests that ketamine-induced locomotion declines with increasing age in male rats and stays stable in female rats.

The neurobiological bases for these age- and sex-dependent differences are unclear. Among the possible explanations are ontogenetic and sex-related differences in NMDA receptor binding site densities (Cyr et al., 2001; Insel et al., 1990) or dopamine (DA) release characteristics (Kuperstein et al., 2008; Sell et al., 2000; Walker and Kuhn, 2008). The latter possibility is based on the hypothesis that ketamine stimulates locomotor activity by directly or indirectly activating a dopaminergic mechanism (Can et al., 2016; Hancock and Stamford, 1999; Irifune et al., 1991; Uchihashi et al., 1992; Usun et al., 2013). Alternatively, pharmacokinetic factors may be responsible for the age- and sex-dependent behavioral differences evident after acute ketamine treatment. In adult male rats, peak ketamine concentrations in brain are reached approximately 5–15 min after injection, while the major metabolite, norketamine, peaks soon after (Páleníček et al., 2011; Saland and Kabbaj, 2018; White et al., 1976). The plasma half-life of ketamine ranges between 27 and 68 min depending on dose and route of administration (Moeller et al., 2019; Saland and Kabbaj, 2018; Toki et al., 2018; Williams et al., 2019), while brain half-life is approximately 14 min (Saland and Kabbaj, 2018). Despite the absence of correlational analyses, there is some evidence that the ketamine-induced locomotor activity of adult male rats is closely associated with brain ketamine concentrations (Páleníček et al., 2011).

Few studies have examined whether ketamine pharmacokinetics varies according to age and sex, but Waterman and Livingston (1978) did find that plasma ketamine levels were significantly elevated in one- and two-week-old rats when compared to adults. Sex differences in ketamine pharmacokinetics have also been reported. When injected with a single low dose of ketamine (2.5 mg/kg), peak brain concentrations of both ketamine and norketamine were significantly elevated in adult female rats relative to males (Saland and Kabbaj, 2018). Saland and Kabbaj (2018) attributed these variations in peak brain concentrations to sex-dependent differences in clearance rates and half-life. Other non-competitive NMDA receptor antagonists exhibit similar properties, as adult female rats metabolize PCP at a slower rate than male rats and show a more prolonged behavioral response to the drug (Nabeshima et al., 1984a, 1984b; Shelnutt et al., 1999; Wessinger, 1995).

The purpose of the present study was to assess the locomotor activating effects of ketamine in male and female rats during three ontogenetic periods: the late preweanling period [postnatal day (PD) 20], early and middle adolescence (PD 30 and PD 40), and adulthood (PD 80). Ketamine (80 mg/kg) was administered at a standard anesthetic dose (Dodelet-Devillers et al., 2016; Giroux et al., 2016), in order to examine the complete cycle of locomotor responsiveness across a 280-min testing session (i.e., from initial ataxia through the onset, progressive rise, and subsequent decline of locomotion; see also Irifune et al., 1991). In a separate experiment, ketamine and norketamine pharmacokinetics were assessed in the dorsal striatum of male and female PD 20, PD 30, PD 40, and PD 80 rats. The relationship between locomotor activity and dorsal striatal ketamine/norketamine levels was characterized using curvilinear regression. To determine whether the pharmacokinetic properties of ketamine differ

between brain regions, hippocampal sections were assayed for ketamine and norketamine. Lastly, because ketamine has been reported to increase dorsal striatal DA content (Irifune et al., 1991; Verma and Moghaddam, 1996; Witkin et al., 2016), the same brain samples used to determine ketamine pharmacokinetics were assayed for DA.

2. Experimental procedures

2.1. Animals

Subjects were 698 male and female Sprague-Dawley rats. Rats tested on PD 80 (males, $N = 96$; females, $N = 106$) were purchased from Charles River (Hollister, CA, USA). Adult rats were allowed to acclimate to the California State University, San Bernardino (CSUSB) vivarium for a minimum of 14 days before testing. Rats tested on PD 20 (males, $N = 96$; females, $N = 96$), PD 30 (males, $N = 56$; females, $N = 56$), or PD 40 (males, $N = 96$; females, $N = 96$) were born and bred at CSUSB. Litters were culled to 10 pups on PD 3 and weaned on PD 21. After weaning, rats were group housed with same-sex littermates. Food and water were freely available. The colony room was maintained at 22–23 °C and kept under a 12:12 h light-dark cycle. Subjects were cared for according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2010) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

2.2. Apparatus

Behavioral testing was done in locomotor activity monitoring chambers that consisted of acrylic walls, a plastic floor, and an open top (Coulbourn Instruments, Whitehall, PA, USA). In order to equate for differences in body size (see also Campbell et al., 1969; Shalaby and Spear, 1980), PD 20 rats were tested in smaller chambers (26 × 26 × 41 cm) than the three older age groups (41 × 41 × 41 cm). Each locomotor activity chamber included an *X-Y* photobeam array, with 16 photocells and detectors, that had a photobeam resolution of either 0.76 cm (small chambers) or 1.27 cm (large chambers). The position of each rat was determined every 100 ms, thus allowing for a precise measure of how much distance (cm) the rat traveled (locomoted) during each 10-min time block.

2.3. Drugs

For the behavioral and pharmacokinetic experiments, (±)-ketamine hydrochloride was dissolved in saline and injected intraperitoneally (i.p.) at a volume of 2.5 ml/kg (PD 20) or 1 ml/kg (PD 30–PD 80). Desipramine hydrochloride (Gross et al., 1999) and (−)-cocaine hydrochloride were used as internal controls in the pharmacokinetic assays, whereas dopamine hydrochloride was the standard in the DA assays. Ketamine was purchased from Spectrum Chemicals (New Brunswick, NJ, USA), while all other compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Behavioral procedures

On the habituation day, which occurred on PD 19, PD 29, PD 39, or PD 79, rats were injected (i.p.) with saline and placed in activity chambers for 30 min. On the test day, which occurred 24 h later (i.e., on PD 20, PD 30, PD 40, or PD 80), rats were injected (i.p.) with saline or 80 mg/kg ketamine immediately before being placed in the activity chambers for 280 min. Younger age groups were not tested because NMDA antagonists, including ketamine, cause apoptotic cell loss in the brains of neonatal rats (Turner et al., 2012).

2.5. HPLC procedures

2.5.1. Injection parameters

On PD 20, PD 30, PD 40, or PD 80, male and female rats were injected (i.p.) with 80 mg/kg ketamine and returned to their home cages. After various time intervals (0, 2.5, 5, 10, 20, 40, 70, 120, 240, or 360 min), rats were killed by rapid decapitation and dorsal striatal and hippocampal sections were dissected bilaterally on an ice-cold dissection plate and stored at -80 °C.

2.5.2. DA content assays

Frozen dorsal striatal and hippocampal samples were sonicated in 200 μl of acetonitrile with 30 μl desipramine hydrochloride (300 ng) and 30 μl (−)-cocaine hydrochloride (3 μg) added as internal standards. Tissue was centrifuged at 20,000 × *g* for 15 min at 4 °C. Twenty microliters of the resulting extracts were assayed for DA using high-performance liquid chromatography (HPLC) with electrochemical detection [Hypersil ODS column (150 × 3 mm) and Coulochem III detector; Thermo Fisher Scientific, Waltham, MA, USA]. The mobile phase consisted of 75 mM NaH_2PO_4, 1.4 mM 1-octane sulfonic acid, 10 mM EDTA, and 10% acetonitrile (pH 3.1) and was pumped at a rate of 0.5 ml/min.

2.5.3. Ketamine and norketamine assays

To measure ketamine and norketamine, the supernatant remaining from the DA content assays was mixed with 350 ml of chloroform:ethanol (4:1) and 50 ml 0.1 M $NaHCO_3$ and centrifuged again for 15 min (Gulley et al., 2003). The top aqueous layer was removed and the lower organic layer was allowed to dry. When completely dry, 200 μl of the mobile phase (0.1 M KH_2PO_4 and 25% acetonitrile, pH 7.0) was added and 100 μl of the resulting solution was injected into the HPLC system (Alliance HPLC system with a 2489 UV detector, Waters, Milford, MA, USA). A Hypersil ODS column (150 mm × 4.6 mm) was used for the separation; the flow rate was set at 1 ml/min and absorbance was monitored at 215 nm.

2.6. Data analysis

For the behavioral experiment, distance traveled data were analyzed using repeated measures analyses of variance (ANOVAs). When the assumption of sphericity was violated, as determined by Mauchly's test of sphericity, the

Greenhouse-Geisser epsilon statistic was used to adjust degrees of freedom (Geisser and Greenhouse, 1958). Corrected degrees of freedom were rounded to the nearest whole number and are indicated by a superscripted "a" in the parenthetical statistical reports. To minimize litter effects, no more than one subject per litter was assigned to a given group (Holson and Pearce, 1992).

For the ketamine and norketamine pharmacokinetic data, as well as the DA content analysis, separate Age × Sex ANOVAs were used. PKSolver (version 2.0; Zhang et al., 2010), employing a non-compartmental model and linear trapezoidal method, was used to determine maximum drug/metabolite concentration (C_{max}), time of maximum concentration (T_{max}), elimination constant (Lambda_Z; 1/min), half-life ($t_{1/2}$), and area under the curve (AUC; 0-360 min). When appropriate, post hoc analysis of behavioral, DA content, and pharmacokinetic data was done using Tukey tests.

Curvilinear regression was used to assess the relationship between dorsal striatal ketamine or norketamine levels, which were measured at various time points (5, 10, 20, 40, 70, 120, 240, and 360 min) after ketamine injection, and distance traveled scores of rats tested for 5 min at the same time points. These analyses utilized group means from separate sets of rats, rather than scores from individual subjects, because rats euthanized in the pharmacokinetic experiment did not provide behavioral data. For the curvilinear regression analyses, linear, quadratic, and cubic models were assessed. In all cases, regression values, main effects, interactions, and post hoc tests were considered significant at $p < 0.05$.

3. Results

3.1. Locomotor activity

3.1.1. Intra-age comparisons

An omnibus ANOVA showed that age interacted with sex and drug to significantly affect distance traveled scores [Age × Sex × Drug interaction $F(3,112) = 4.08$, $p < 0.01$; ^{a}Age × Sex × Drug × TB interaction $F(14,532) = 3.47$, $p < 0.001$]. Among PD 20 rats (see Fig. 1, upper graph), 80 mg/kg ketamine caused a significant increase in distance traveled, relative to the saline group, on TB 5-23 [Drug main effect $F(1,28) = 274.56$, $p < 0.001$; ^{a}Drug × TB interaction $F(3,77) = 42.35$, $p < 0.001$]. Locomotor activity did not vary according to sex at PD 20.

At the three older ages, 80 mg/kg ketamine induced more locomotor activity than saline, and this effect was significantly more pronounced in female rats than male rats (see Fig. 1) [Sex × Drug interactions $F(1,28) = 8.41$, $p < 0.01$; $F(1,28) = 12.25$, $p < 0.01$; $F(1,28) = 21.42$, $p < 0.01$, respectively]. The time frame in which ketamine caused peak locomotor activity varied according to age and sex. Among PD 30 rats, ketamine-treated male rats exhibited greater distance traveled scores than their saline controls towards the start of the session (i.e., on TB 3-8); whereas, ketamine-treated female rats exhibited more distance traveled than their saline controls on TB 5-21 [^{a}Sex × Drug × TB interaction $F(3,71) = 7.84$, $p < 0.001$]. Direct comparisons between male and female PD 30 rats showed that ketamine-treated male rats had greater distance traveled scores than similarly treated females towards the start of the session (TB 3 and 4), while ketamine produced greater distance traveled in female PD 30 rats than male rats on TB 7-14 [Tukey tests, $p < 0.05$].

Sex differences in ketamine-induced locomotor activity were even more apparent in PD 40 rats (see Fig. 1). Ketamine-treated male PD 40 rats had greater distance traveled scores than their saline controls on TB 2-6, while ketamine-treated female PD 40 rats exhibited more locomotor activity than their saline controls later in the testing session (i.e., on TB 6-22) [^{a}Sex × Drug × TB interaction $F(5,133) = 18.94$, $p < 0.001$]. Distance traveled scores of ketamine-treated male PD 40 rats were elevated relative to females on TB 2-5, while female scores were greater than male scores on TB 7-21 [Tukey tests, $p < 0.05$]. In male PD 80 rats, ketamine only increased distance traveled scores on TB 4 and 5 [^{a}Sex × Drug × TB interaction $F(5,134) = 11.00$, $p < 0.001$]. In contrast, ketamine-treated female PD 80 rats had greater distance traveled scores on TB 9-27 than female saline controls and ketamine-treated male rats [Tukey tests, $p < 0.05$].

3.1.2. Inter-age comparisons

Comparisons across age showed that locomotor activity differed in ketamine-treated male and female rats, but not in saline-treated rats (i.e., saline-treated rats exhibited low levels of locomotor activity regardless of age) [Age × Sex × Drug interaction $F(3,112) = 4.08$, $p < 0.01$; ^{a}Age × Sex × Drug × TB interaction $F(14,532) = 3.47$, $p < 0.001$]. Separate ANOVAs assessing only the ketamine-treated rats showed that male PD 20 rats had greater distance traveled scores than male PD 80 rats, whereas ketamine-treated female PD 20 rats responded similarly to female PD 80 rats (see Fig. 1) [Age × Sex interaction $F(3,56) = 5.25$, $p < 0.01$]. These effects varied across the testing session, as male PD 20 rats exhibited more distance traveled on TB 8-18 than male PD 80 rats [^{a}Age × Sex × TB interaction $F(11,209) = 4.14$, $p < 0.001$]. The responding of female rats peaked at different time points, since ketamine-treated female PD 20 rats exhibited more distance traveled than female PD 80 rats on TB 7 and 8, but PD 80 rats had greater distance traveled scores than PD 20 rats on TB 18-23 and TB 25-27 [Tukey tests, $p < 0.05$].

When comparing PD 30 to PD 80 rats, distance traveled scores of ketamine-treated male rats did not differ according to age. On the other hand, the distance traveled scores of female PD 30 rats peaked earlier in the testing session than PD 80 rats (see Fig. 1). Specifically, ketamine-treated female PD 30 rats had greater distance traveled scores than female PD 80 rats on TB 5-9. This age effect was reversed on TB 14-28, with PD 80 rats showing greater ketamine-induced locomotor activity [Tukey tests, $p < 0.05$]. Comparisons between PD 40 and PD 80 rats were similar to those just described, as ketamine did not differentially affect the locomotor activity of male rats. Ketamine-treated female PD 40 rats exhibited less distance traveled than female PD 80 rats, with the younger female rats showing less locomotion on TB 14-23, 25, and 27 [Tukey tests, $p < 0.05$].

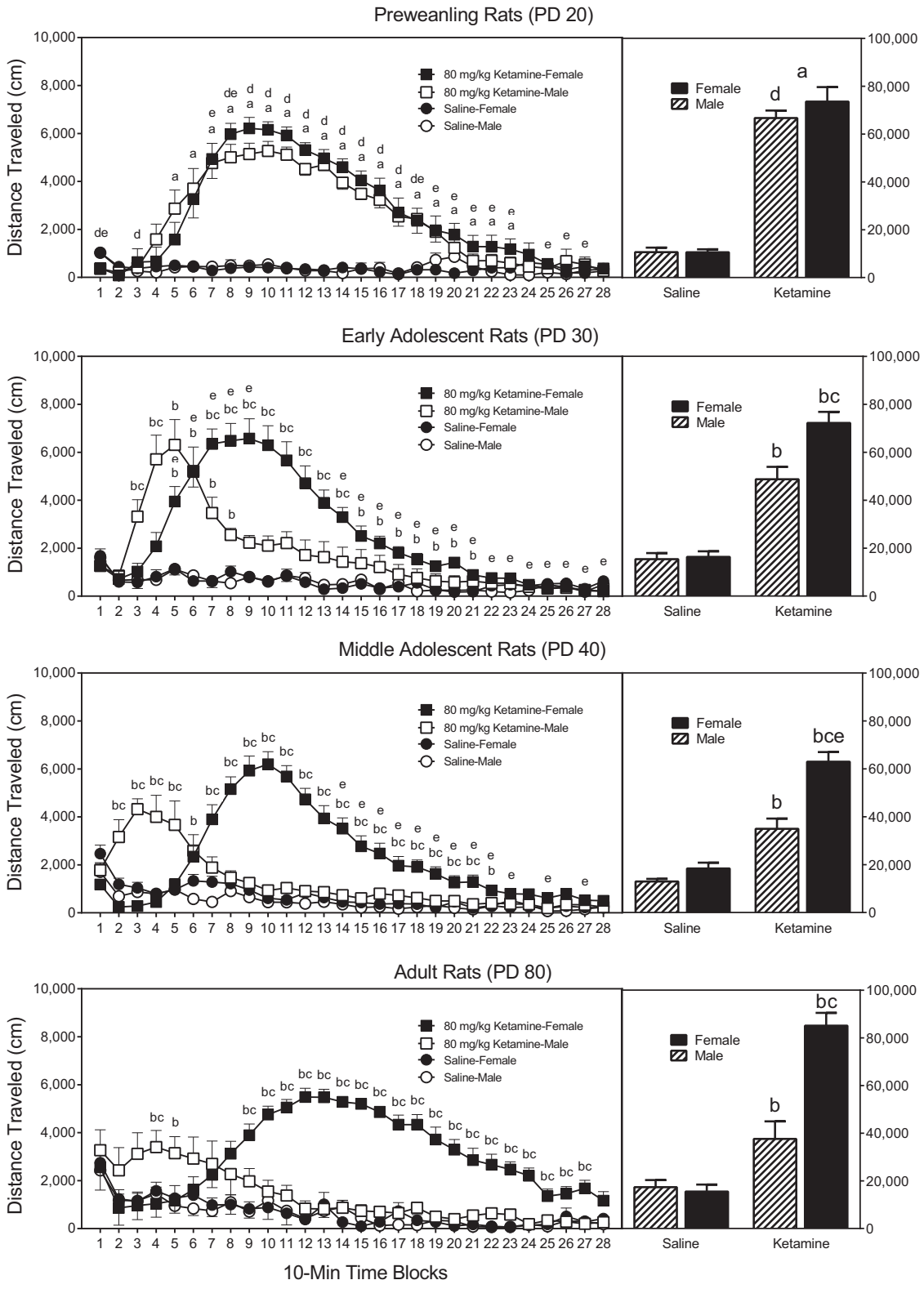

Fig. 1 Mean (±SEM) distance traveled scores of male and female rats ($n = 8$ rats per group) on the test day. Preweanling (PD 20), adolescent (PD 30 and PD 40), and adult rats (PD 80) were injected with saline or ketamine (80 mg/kg, i.p.) immediately before testing. The right graphs show data collapsed across the 28 10-min time blocks. 'a' Significantly different from same-age rats given saline; 'b' significantly different from same-age/same-sex rats given saline; 'c' significantly different from same-age/opposite-sex rats given 80 mg/kg ketamine; 'd' significantly different from male adult (PD 80) rats given 80 mg/kg ketamine; 'e' Significantly different from female adult (PD 80) rats given 80 mg/kg ketamine.

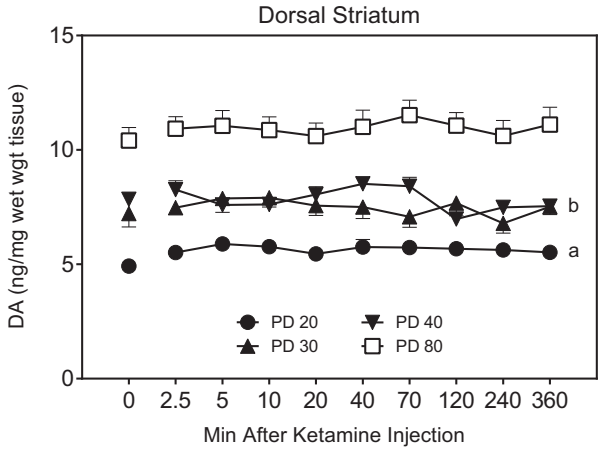

Fig. 2 Mean (±SEM) DA levels in the dorsal striatum of preweanling (PD 20, $n = 8$ per group), adolescent (PD 30 and PD 40) and adult rats (PD 80). Brains were removed 0-360 min after ketamine (80 mg/kg, i.p.) treatment. 'a' Significantly different from all other age groups. 'b' Significantly different from PD 80 rats.

3.2. Dorsal striatal DA content

Among the four age groups, DA content in the dorsal striatum was greatest in adult rats and lowest in preweanling rats, with adolescent rats having DA levels intermediate between, and significantly different from, the younger and older age groups (see Fig. 2) [Age main effect $F(3,33) = 18.41$, $p < 0.001$]. Dorsal striatal DA levels were not elevated at any time point (2.5-360 min) after ketamine treatment, nor did DA levels differ according to sex. DA was not reliably detected in the hippocampus (see also Dellu-Hagedorn et al., 2017; Fitoussi et al., 2013).

3.3. Ketamine pharmacokinetics

3.3.1. Maximum ketamine concentrations

In both the dorsal striatum and hippocampus (see Tables 1 and 2), the maximum concentration of ketamine (C_{max}) was greater in female rats than male rats [Sex main effects $F(1,47) = 7.18$, $p < 0.01$; $F(1,47) = 16.83$, $p < 0.001$], but this effect interacted with age. Specifically, C_{max} values of female rats did not vary according to age; whereas, C_{max} values of male PD 40 and PD 80 rats were significantly reduced relative to male PD 20 rats and age-matched female rats [Sex × Age interactions $F(3,47) = 5.47$, $p < 0.01$; $F(3,47) = 7.32$, $p < 0.001$]. Time until maximum ketamine concentrations (T_{max}) in the dorsal striatum ($\bar{x} = 6.9$ min) and hippocampus ($\bar{x} = 7.2$ min) did not vary significantly according to age or sex.

3.3.2. Ketamine elimination

Regardless of brain region, the ketamine elimination constant (Lambda_Z) was larger in males than females (see Fig. 3) [Sex main effects $F(1,47) = 4.10$, $p < 0.05$; $F(1,47) = 46.94$, $p < 0.001$]. In the dorsal striatum (see Table 1), Lambda_Z values were greater in PD 40 rats than PD 20 rats [Age main effect $F(3,47) = 4.10$, $p < 0.05$]. In the hippocampus (see Table 2), Lambda_Z values of female rats did not differ according to age; whereas, male PD 30 and PD 40 rats had larger elimination constants than younger and older male rats [Sex × Age interaction $F(3,47) = 8.07$, $p < 0.001$]. Only at PD 40, were the Lambda_Z values of female rats significantly reduced relative to male rats [Tukey tests, $p < 0.05$].

Ketamine half-life ($t_{1/2}$) varied according to brain area, as only in the hippocampus were the $t_{1/2}$ values of ketamine-treated female rats greater than male rats [Sex main effect $F(1,47) = 29.62$, $p < 0.001$]. In the dorsal striatum, ketamine $t_{1/2}$ of PD 40 rats ($\bar{x} = 34.8$ min) was reduced relative to PD 20 ($\bar{x} = 59.6$ min) and PD 80 ($\bar{x} = 58.5$ min) rats [Age main effect $F(3,47) = 6.05$, $p < 0.01$]; whereas, hippocampal $t_{1/2}$ values of PD 30 ($\bar{x} = 37.2$ min) and PD 40 ($\bar{x} = 36.1$ min) rats were shorter than those measured in PD 20 ($\bar{x} = 50.5$ min) and PD 80 ($\bar{x} = 51.9$ min) rats [Age main effect $F(3,47) = 9.78$, $p < 0.001$].

3.3.3. Ketamine availability

In both the dorsal striatum (see Table 1) and hippocampus (see Table 2), AUC, which is a measure of drug availability, was greater in female rats than male rats [Sex main effects $F(1,47) = 28.59$, $p < 0.001$; $F(1,47) = 55.62$, $p < 0.001$]. AUC was also greater in preweanling rats (PD 20) than older rats (PD 30-PD 80) [Age main effects $F(3,47) = 21.66$, $p < 0.001$; $F(3,47) = 36.02$, $p < 0.001$]. Analysis of the two Sex × Age interactions indicated that the AUC of female rats did not vary according to age in either brain region. In contrast, male preweanling rats had greater AUC values than older male rats in both the dorsal striatum and hippocampus [$F(3,47) = 5.51$, $p < 0.01$; $F(3,47) = 14.36$, $p < 0.001$]. AUC of ketamine-treated male rats was reduced relative to female rats on PD 40 and PD 80 (dorsal striatum) and PD 30, PD 40, and PD 80 (hippocampus) [Tukey tests, $p < 0.05$].

3.3.4. Recovery of standards

In terms of male and female rats, recovery of (−)-cocaine hydrochloride in the dorsal striatum (75.8% and 74.8%, respectively) and hippocampus (81.1% and 79.9%, respectively) were similar to literature values (Gross et al., 1999; Li et al., 2012). Recovery of desipramine hydrochloride provided results similar to cocaine.

3.4. Relationship between ketamine levels in the dorsal striatum and distance traveled scores

Among male rats, the relationship between dorsal striatal ketamine levels and distance traveled was best fit by a cubic model (see Fig. 5, left panels). At all ages, the relationship between ketamine levels and locomotor activity appeared as an inverted U-shaped function, with three of the age groups showing an additional positive deflection at the extreme upper end [PD 20, $F(3,4) = 7.43$, $p < 0.05$; PD 30, $F(3,4) = 8.76$, $p < 0.05$; PD 40, $F(3,4) = 12.66$, $p < 0.05$; PD 80, $F(3,4) = 75.02$, $p < 0.001$]. The R-squared (r^2) values for male rats varied between 0.85 and 0.98 depending on age.

Table 1 Ketamine pharmacokinetics in the dorsal striatum of male and female preweanling, adolescent, and adult rats injected with 80 mg/kg ketamine (i.p.).

Age-sex	Ketamine				
	C_{max}	T_{max}	Lambda_Z	$t_{1/2}$	AUC
PD 20 ($n = 8$ per group)					
Male	79.10 (7.4)	8.12 (1.9)	0.012 (0.0005)	58.66 (2.3)	4211.27 (442.1)
Female	69.30 (3.6)	7.81 (2.0)	0.012 (0.0008)	60.48 (4.1)	4084.13 (290.5)
PD 30 ($n = 4$ per group)					
Male	64.28 (0.9)	7.50 (1.4)	0.019 (0.0044)	43.21 (9.6)	2236.57 (225.0)[b]
Female	67.37 (2.4)	7.50 (1.4)	0.012 (0.0006)	57.94 (2.6)	3550.32 (243.2)
PD 40 ($n = 8$ per group)					
Male	50.05 (4.2)[b]	7.19 (2.1)	0.025 (0.0022)[c]	29.58 (2.9)[c,d]	1337.27 (80.30)[b]
Female	77.79 (8.2)[a]	7.50 (1.1)	0.017 (0.0018)[c]	41.82 (4.1)[c,d]	2930.35 (260.05)[a]
PD 80 ($n = 8$-9 per group)					
Male	40.74 (3.6)[b]	3.75 (0.5)	0.017 (0.0043)	57.80 (10.8)	1203.61 (129.11)[b]
Female	63.00 (5.2)[a]	6.67 (0.8)	0.014 (0.0033)	59.06 (7.20)	3218.93 (355.51)[a]

C_{max}, maximum concentration of ketamine (μg/g); T_{max}, time of maximum concentration (min after injection); Lambda_Z, elimination constant (1/min); $t_{1/2}$, ketamine half-life (min); AUC, area under the curve (0-360 min).
[a] Significantly different from male rats of the same age.
[b] Significantly different from PD 20 rats of the same sex.
[c] Significantly different from PD 20 rats (age main effect).
[d] Significantly different from PD 80 rats (age main effect).

Table 2 Ketamine pharmacokinetics in the hippocampus of male and female preweanling, adolescent, and adult rats injected with 80 mg/kg ketamine (i.p.).

Age-sex	Ketamine				
	C_{max}	T_{max}	Lambda_Z	$t_{1/2}$	AUC
PD 20 ($n = 8$ per group)					
Male	64.40 (1.9)	9.38 (1.8)	0.015 (0.0010)	47.37 (3.0)	3784.66 (309.5)
Female	64.26 (3.5)	5.62 (0.6)	0.013 (0.0008)	53.56 (3.3)	3565.28 (136.0)
PD 30 ($n = 4$ per group)					
Male	55.50 (3.7)	7.50 (1.4)	0.028 (0.0040)[b,c]	26.28 (4.6)[d,e]	1731.62 (284.9)[b]
Female	52.89 (3.0)	8.75 (1.2)	0.025 (0.0154)	48.16 (6.5)[d,e]	2712.99 (146.5)[a]
PD 40 ($n = 8$ per group)					
Male	41.93 (4.4)[b]	8.75 (2.0)	0.028 (0.0016)[b,c]	25.71 (1.6)[d,e]	1242.63 (85.0)[b]
Female	66.24 (4.4)[a]	7.14 (1.0)	0.015 (0.0009)[a]	47.90 (2.5)[d,e]	2791.13 (208.2)[a]
PD 80 ($n = 8$-9 per group)					
Male	32.17 (3.9)[b]	5.94 (2.0)	0.016 (0.0015)	47.46 (4.6)	901.42 (104.2)[b]
Female	56.57 (3.5)[a]	5.31 (0.7)	0.013 (0.0010)	56.40 (4.0)	3054.88 (224.2)[a]

C_{max}, maximum concentration of ketamine (μg/g); T_{max}, time of maximum concentration (min after injection); Lambda_Z, elimination constant (1/min); $t_{1/2}$, ketamine half-life (min); AUC, area under the curve (0-360 min).
[a] Significantly different from male rats of the same age.
[b] Significantly different from PD 20 rats of the same sex.
[c] Significantly different from PD 80 rats of the same sex.
[d] Significantly different from PD 20 rats (age main effect).
[e] Significantly different from PD 80 rats (age main effect).

Among female rats (see Fig. 5, right panels), the relationship between ketamine levels and distance traveled scores in the two youngest age groups [PD 20, $F(3,4) = 4.13$, $p = 0.10$, $r^2 = 0.76$; PD 30, $F(3,4) = 8.35$, $p < 0.05$, $r^2 = 0.86$] were almost identical to male rats (i.e., the data were best fit by a cubic model and the r^2 values were large). Although a cubic model was still the best descriptor of the relationship between these variables in PD 40 ($r^2 = 0.58$) and PD 80 ($r^2 = 0.53$) female rats, the relationships were nonsignificant and the r^2 values were comparatively small.

3.5. Norketamine pharmacokinetics

3.5.1. Maximum norketamine concentrations

In both the dorsal striatum and hippocampus (see Tables 3 and 4), norketamine C_{max} values were significantly elevated in female rats relative to males [Sex main effects $F(1,50) = 5.21$, $p < 0.05$; $F(1,48) = 10.35$, $p < 0.01$], but this effect was only apparent at PD 40 and PD 80 [Sex × Age interactions $F(3,50) = 5.03$, $p < 0.05$; $F(3,48) = 3.47$, $p < 0.05$]. In both brain regions, the

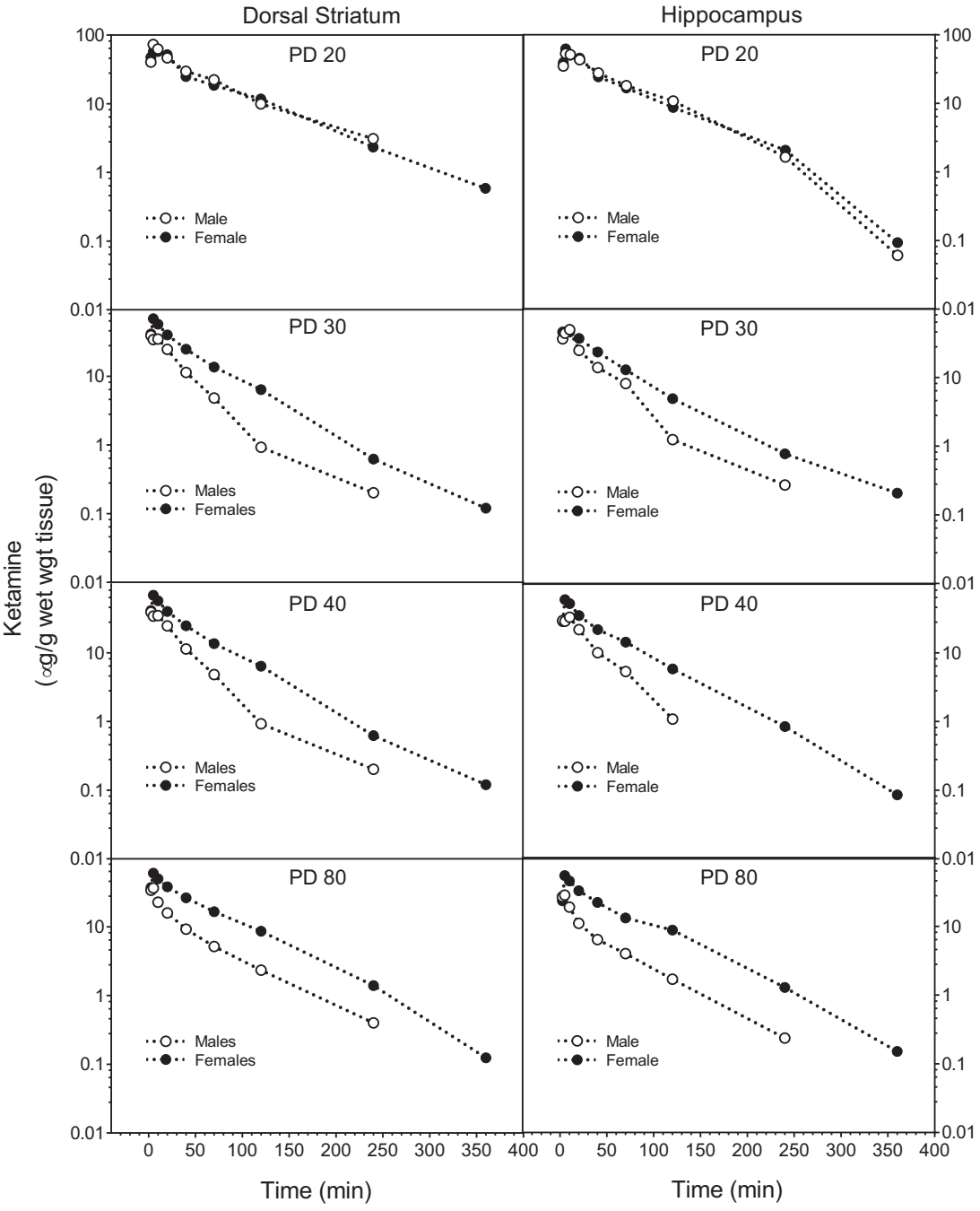

Fig. 3 Nonlinear regression showing the ketamine concentration-time curves for male and female rats injected with ketamine (80 mg/kg, i.p.) at different postnatal ages. Discontinuation of the concentration-time curves indicates that ketamine levels were zero at subsequent time points.

norketamine T_{max} values of female rats did not vary according to age; whereas, T_{max} was shorter in male PD 40 and PD 80 rats relative to male PD 20 rats and age-matched female rats [Sex × Age interactions $F(3,50) = 3.07$, $p < 0.05$; $F(3,48) = 5.80$, $p < 0.01$].

3.5.2. Norketamine elimination

In both brain regions, Lambda_Z was smaller in female rats than male rats (see Fig. 4) [Sex main effects $F(1,50) = 17.85$, $p < 0.001$; $F(1,47) = 28.11$, $p < 0.001$], but only in the dorsal striatum was this sex difference restricted to PD 40 and PD 80 rats (see Table 3) [Sex × Age interaction $F(3,50) = 6.08$, $p < 0.01$]. In the hippocampus (see Table 4), the elimination constant was significantly elevated in the two adolescent age groups relative to adult rats [Age main effect $F(3,47) = 4.56$, $p < 0.01$].

Consistent with the Lambda_Z data, norketamine $t_{1/2}$ was longer in female rats than male rats in both the dorsal striatum and hippocampus (see Tables 3 and 4) [Sex main effects $F(1,50) = 10.49$, $p < 0.01$; $F(1,47) = 22.69$, $p < 0.001$].

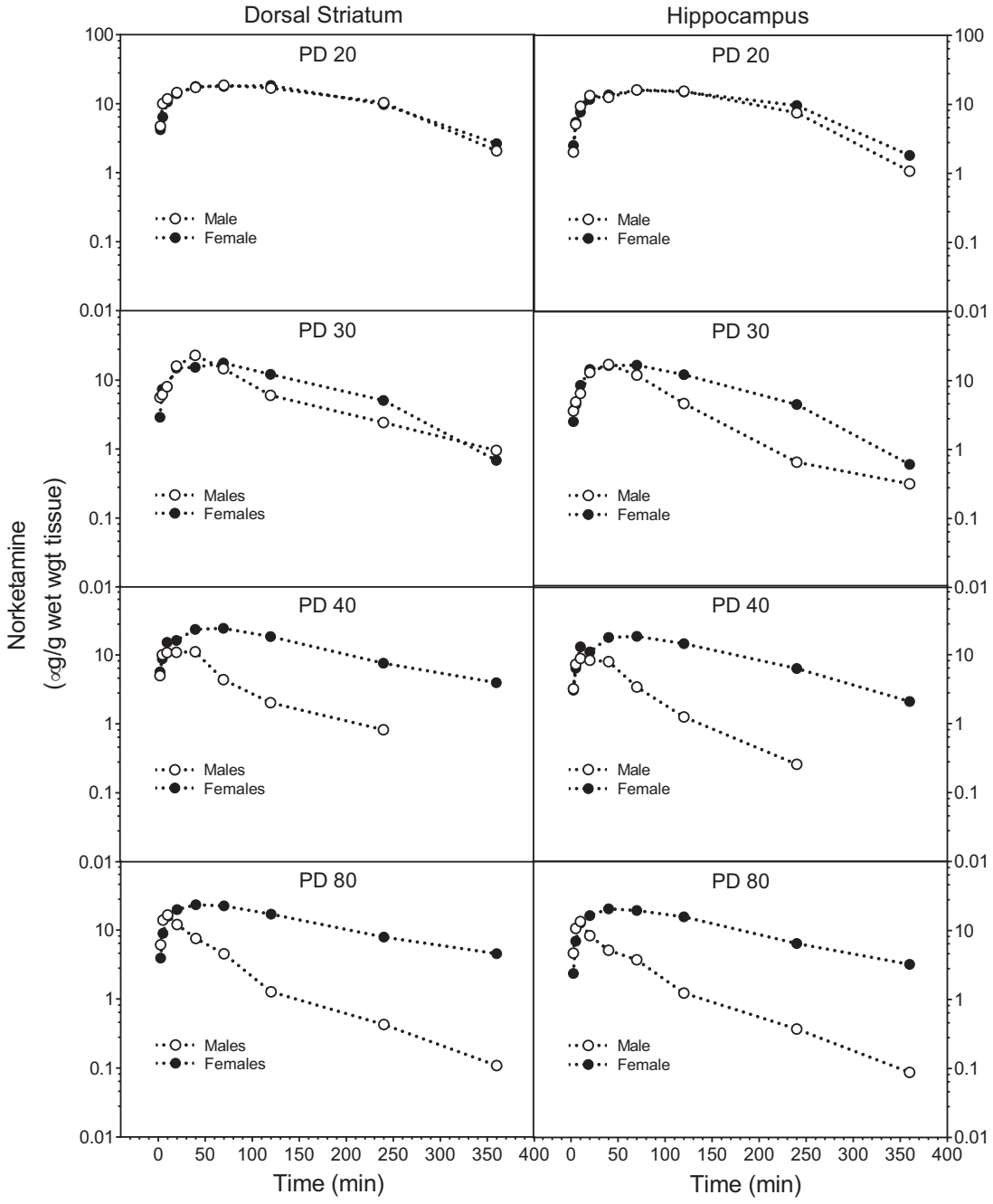

Fig. 4 Nonlinear regression showing the norketamine concentration-time curves for male and female rats injected with ketamine (80 mg/kg, i.p.) at different postnatal ages.

In both brain regions, these sex differences in norketamine $t_{1/2}$ were limited to PD 40 and PD 80 rats [[Sex × Age interactions $F(3,50) = 3.61$, $p < 0.05$; $F(3,48) = 2.85$, $p < 0.05$]. Only in the hippocampus were the $t_{1/2}$ values of female PD 20 and PD 30 rats significantly shorter than female PD 80 rats Tukey tests, $p < 0.05$].

3.5.3. Norketamine availability

In both brain regions (see Tables 3 and 4), norketamine AUC of female rats did not vary according to age; whereas, male PD 20 rats had significantly larger AUC values than male PD 40 and PD 80 rats (dorsal striatum) and male PD 30, PD 40, and PD 80 rats (hippocampus) [Sex × Age interactions $F(3,50) = 15.62$, $p < 0.001$; $F(3,48) = 11.81$, $p < 0.001$]. Regardless of brain area, the AUC values of male PD 40 and PD 80 rats were smaller than female rats of the same age [Tukey tests, $p < 0.05$].

3.6. Relationship between norketamine levels in the dorsal striatum and distance traveled scores

In male rats, cubic functions best describe the relationship between dorsal striatal norketamine levels and distance

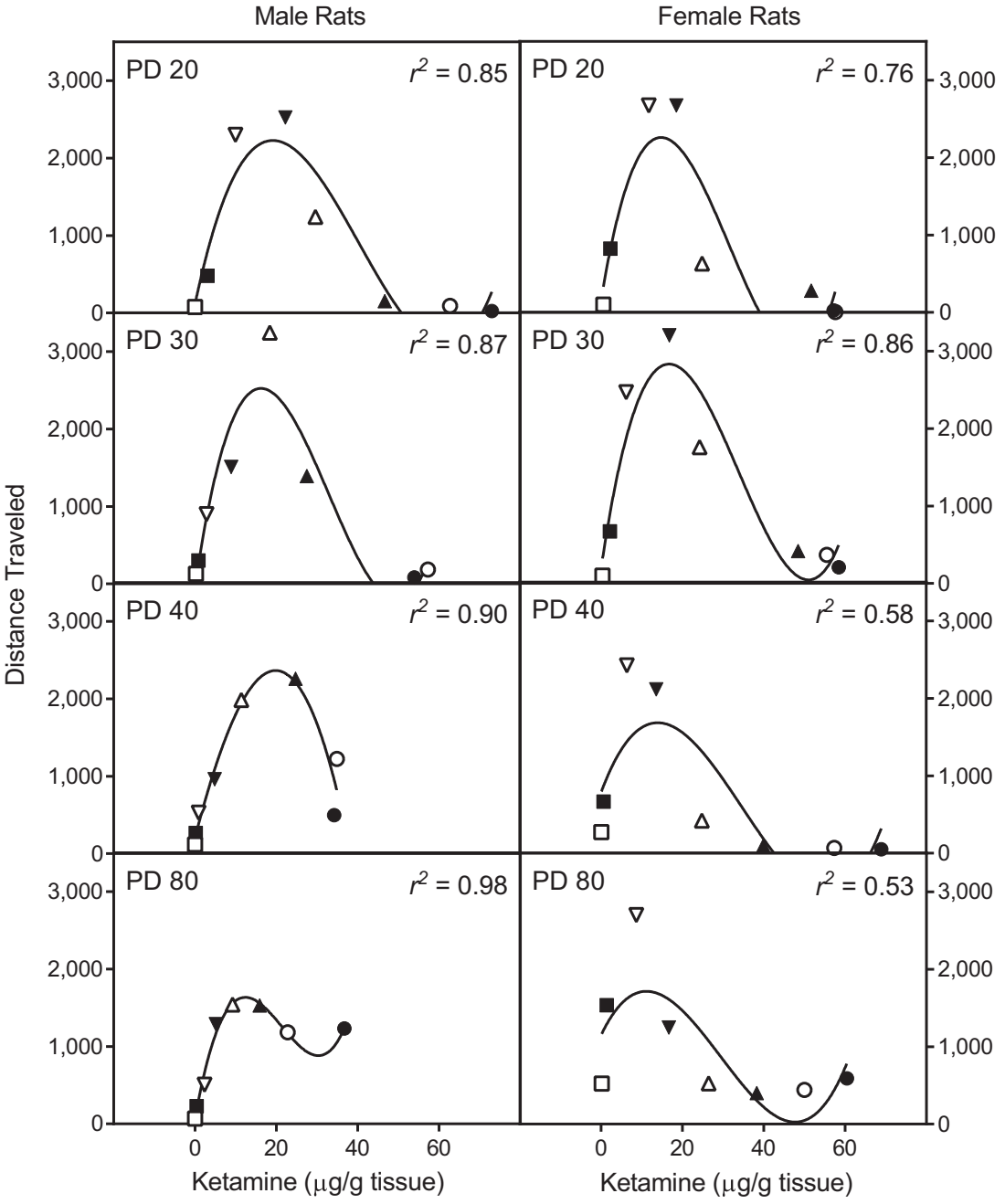

Fig. 5 Scatterplots representing the relationship between dorsal striatal ketamine concentrations and the distance traveled scores of male and female rats on PD 20, PD 30, PD 40, and PD 80. Each point represents the mean scores of separate groups of male and female rats tested 5, 10, 20, 40, 70, 120, 240, and 360 min after ketamine (80 mg/kg, i.p.) treatment. The regression line was determined using the model with the best fit. Filled circles = 5 min; open circles = 10 min; filled triangles = 20 min; open triangle = 40 min; filled inverted triangles = 70 min; open inverted triangles = 120 min; filled squares = 240 min; and open squares = 360 min.

traveled (see Fig. 6, left panels). In PD 20 [$F(3,4) = 7.27$, $p < 0.05$], PD 30 [$F(3,4) = 18.64$, $p < 0.01$], and PD 40 [$F(3,4) = 5.73$, $p = 0.06$] male rats, the relationship between these two variables is best depicted by an accelerating upward curve. In PD 80 male rats [$F(3,4) = 197.58$, $p < 0.001$], the accelerating function deflects at the upper end (i.e., very high norketamine levels are correlated with a gradual decrease in locomotion). r^2 values of male rats varied between 0.84 and 0.99 depending on age.

In PD 20 and PD 30 female rats, there was a positive linear relationship between distance traveled scores and dorsal striatal norketamine levels (see Fig. 6, right panels) [$F(1,6) = 5.99$, $p < 0.05$, $r^2 = 0.50$; $F(1,6) = 6.23$, $p < 0.05$, $r^2 = 0.51$, respectively]. In older female rats (PD 40 and PD 80), the correlation between distance traveled and norke-

Table 3 Norketamine pharmacokinetics in the dorsal striatum of male and female preweanling, adolescent, and adult rats injected with 80 mg/kg ketamine (i.p.).

Age-sex	Norketamine				
	C_{max}	T_{max}	Lambda_Z	$t_{1/2}$	AUC
PD 20 ($n = 8$ per group)					
Male	19.55 (0.9)	77.5 (13.2)	0.007 (0.0009)	101.7 (12.1)	4188.0 (376.3)
Female	19.92 (0.8)	74.4 (12.7)	0.008 (0.0008)	93.60 (9.6)	4372.9 (153.6)
PD 30 ($n = 4$ per group)					
Male	23.20 (1.1)	35.0 (5.0)[b]	0.011 (0.0027)	52.18 (2.9)	2206.2 (201.6)
Female	18.76 (4.0)	57.5 (12.5)	0.008 (0.0005)	83.80 (5.0)	3931.4 (443.9)
PD 40 ($n = 8$ per group)					
Male	17.34 (2.4)	21.2 (5.7)[b]	0.014 (0.0019)	57.96 (7.2)	931.71 (174.7)[b]
Female	25.99 (2.0)[a]	55.0 (5.7)[a]	0.007 (0.0006)[a]	104.6 (9.3)[a]	4665.0 (392.5)[a]
PD 80 ($n = 8$-9 per group)					
Male	17.36 (1.4)	11.2 (2.0)[b]	0.017 (0.0028)[b]	49.79 (8.9)	870.60 (88.64)[b]
Female	24.54 (1.3)[a]	56.7 (5.3)[a]	0.005 (0.0007)[a]	158.7 (31.6)[a]	4445.2 (504.2)[a]

C_{max}, maximum concentration of norketamine (μg/g); T_{max}, time of maximum concentration (min after injection); Lambda_Z, elimination constant (1/min); $t_{1/2}$, norketamine half-life (min); AUC, area under the curve (0-360 min).
[a] Significantly different from male rats of the same age.
[b] Significantly different from PD 20 rats of the same sex.

Table 4 Norketamine pharmacokinetics in the hippocampus of male and female preweanling, adolescent, and adult rats injected with 80 mg/kg ketamine (i.p.).

Age-sex	Norketamine				
	C_{max}	T_{max}	Lambda_Z	$t_{1/2}$	AUC
PD 20 ($n = 8$ per group)					
Male	17.68 (1.7)	85.0 (10.8)	0.011 (0.0012)	70.27 (8.2)	3485.4 (404.2)
Female	17.30 (1.8)	68.8 (12.9)	0.009 (0.0009)	86.42 (11.7)[c]	3710.2 (266.9)
PD 30 ($n = 4$ per group)					
Male	17.22 (2.0)	35.0 (5.0)[b]	0.017 (0.0044)[d]	47.53 (8.2)	1675.4 (448.6)[b]
Female	18.24 (3.0)	50.0 (12.2)	0.011 (0.0001)[d]	64.52 (0.8)[c]	2910.0 (413.4)
PD 40 ($n = 8$ per group)					
Male	13.60 (1.4)	14.4 (4.3)[b]	0.017 (0.0018)[d]	43.10 (4.3)	641.08 (59.41)[b]
Female	20.34 (1.2)[a]	61.25 (9.9)[a]	0.008 (0.0006)[d]	88.02 (7.9)[a]	3591.1 (199.4)[a]
PD 80 ($n = 8$-9 per group)					
Male	14.17 (1.0)	12.5 (1.6)[b]	0.012 (0.0017)	65.71 (8.6)	771.24 (115.3)[b]
Female	22.66 (2.0)[a]	51.2 (5.5)[a]	0.006 (0.0005)	133.1 (16.1)[a]	3605.4 (162.8)[a]

C_{max}, maximum concentration of norketamine (μg/g); T_{max}, time of maximum concentration (min after injection); Lambda_Z, elimination constant (1/min); $t_{1/2}$, norketamine half-life (min); AUC, area under the curve (0-360 min).
[a] Significantly different from male rats of the same age.
[b] Significantly different from PD 20 rats of the same sex.
[c] Significantly different from PD 80 rats of the same sex.
[d] Significantly different from PD 80 rats (age main effect).

tamine levels was small ($r^2 = 0.30$ and $r^2 = 0.11$, respectively) and nonsignificant.

4. Discussion

Only recently have researchers started the process of systematically examining the locomotor activating effects of low to moderate doses of ketamine (3-40 mg/kg) in both sexes (McDougall et al., 2017; Schoepfer et al., 2019; Wilson et al., 2005, 2007) and across early ontogeny (McDougall et al., 2017; Rocha et al., 2017; Wiley et al., 2011; Wilson et al., 2007). In the present study, we administered a standard anesthetic dose of ketamine (80 mg/kg) to male and female preweanling, adolescent, and adult rats in order to examine the full pattern of locomotor responsiveness from initial ataxia through the onset, rise, and eventual decline of ketamine-induced locomotion (see also Irifune et al., 1991). In general, ketamine affected the locomotor activity of female rats similarly across age, as the TB on which peak locomotion occurred varied only modestly among the four female age groups (i.e., with increasing age there was

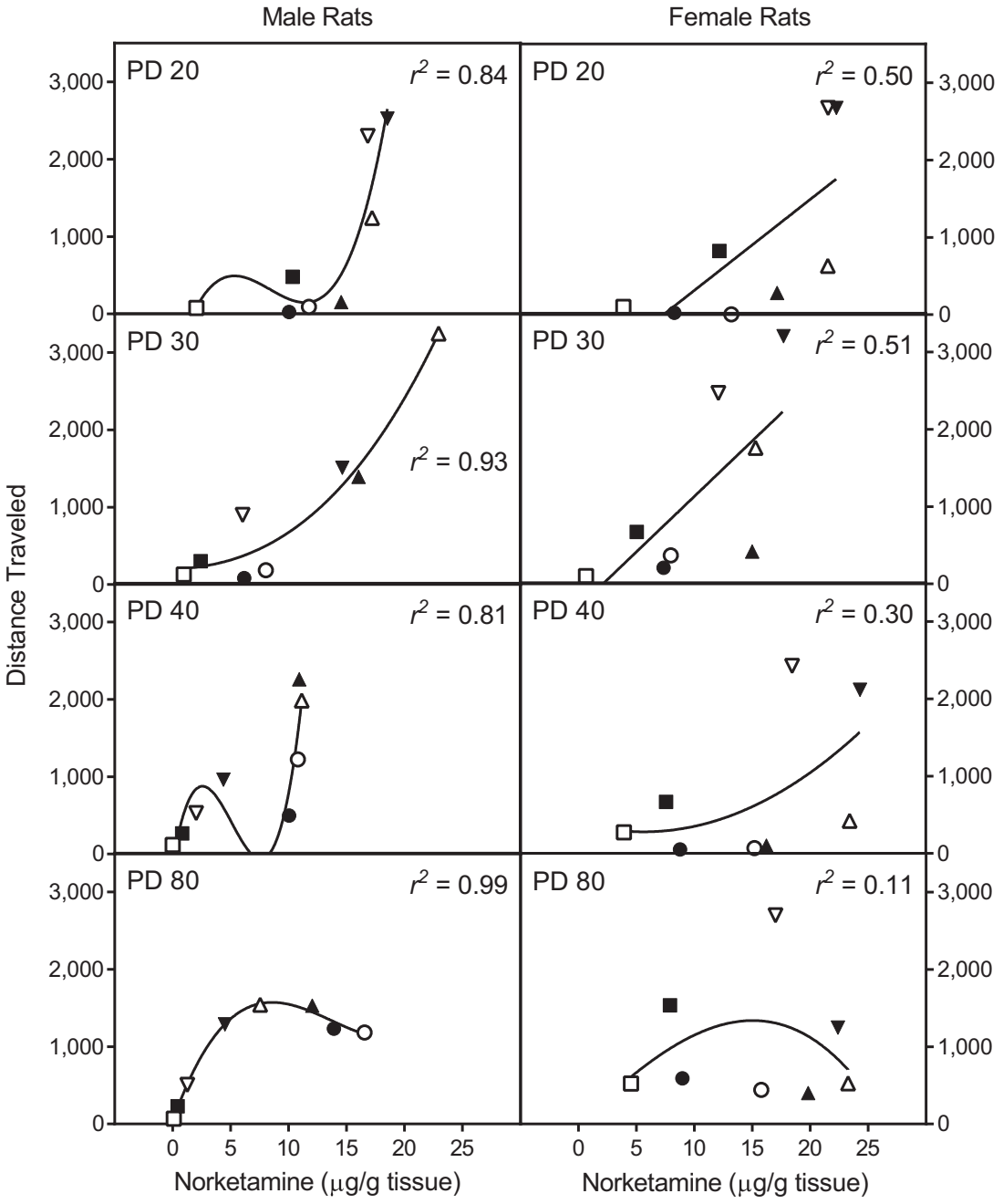

Fig. 6 Scatterplots representing the relationship between dorsal striatal norketamine concentrations and distance traveled scores of male and female rats on PD 20, PD 30, PD 40, and PD 80. Characteristics of the scatterplot are the same as described for Fig. 5.

a slight rightward shift in ketamine's locomotor activating effects; PD 20 and PD 30, TB 9; PD 40, TB 10; PD 80, TB 12). When compared to preweanling rats or age-matched females, adolescent and adult male rats exhibited both: (a) lesser amounts of ketamine-induced locomotor activity, and (b) shorter latencies to reach peak locomotion (PD 30, TB 5; PD 40, TB 3; PD 80, TB 4). In adult rats, for example, males ($\bar{x}$= 3398 cm traveled, TB 4) reached peak locomotor activity levels 80 min before females ($\bar{x}$= 5487 cm traveled, TB 12). Although not described earlier, the motoric capacity of each rat was quantified and all age groups showed a period of immobility, followed by ataxia, at the start of the testing session (see Supplementary Materials). Preweanling rats and older female rats showed longer periods of immobility than male adolescent and adult rats (see also McDougall et al., 2017). Despite this extended period of immobility, adolescent and adult female rats exhibited more total locomotor activity across the 280-min testing session than age-matched male rats. Considering these results as a whole, it appears that a single high-dose injection of ketamine (80 mg/kg) has the greatest behavioral impact on female rats and preweanling rats, and a lesser effect on male adolescent and adult rats.

In many ways, the age- and sex-dependent behavioral differences evident after ketamine treatment are mirrored by changes in ketamine pharmacokinetics. Most notably,

ketamine C_{max} values and AUC, a measure of drug availability, did not vary among the four female age groups; whereas, both C_{max} and AUC were significantly reduced in male PD 40 and PD 80 rats relative to age-matched females. For example, the dorsal striatal C_{max} ($\bar{x}$= 63.00 μg/g) and AUC ($\bar{x}$= 3219 μg/g × time) values of adult female rats were significantly greater than age-matched male rats (C_{max}, $\bar{x}$= 40.74 μg/g; AUC, $\bar{x}$= 1204 μg/g × time). Interestingly, ketamine $t_{1/2}$ ($\bar{x}$= 51.6 min) and T_{max} ($\bar{x}$= 6.9 min) in the dorsal striatum did not differ according to age or sex, indicating that peak ketamine concentrations (C_{max}) and total drug availability (AUC) were the two factors most closely associated with the observed changes in behavior. Similar results were reported by Saland and Kabbaj (2018), as they found that brain C_{max} values of adult female rats were greater than in males, while $t_{1/2}$ and T_{max} did not vary according to sex.

The underlying reason why ketamine absorption and/or metabolism varies according to age and sex is uncertain, but age-dependent changes in ketamine half-life during early and late adulthood have been attributed to liver metabolism (Giroux et al., 2016), probably involving cytochrome P450 enzymes (Veilleux-Lemieux et al., 2013). Consistent with this possibility, the activity of cytochrome P450 enzymes is reduced in female rats relative to male rats, and this difference is responsible for the elevated concentrations of PCP in the brains of adult female rats (Nabeshima et al., 1984a, 1984b). As expected, ketamine pharmacokinetics differed only slightly between brain regions (Saland and Kabbaj, 2018), and the C_{max}, $t_{1/2}$, and T_{max} values of our adult male rats were generally consistent with literature values despite differences in dose, compartment, and route of administration (Páleníček et al., 2011; Saland and Kabbaj, 2018; Toki et al., 2018; White et al., 1976; Williams et al., 2019).

In terms of age and sex effects, norketamine pharmacokinetics differed substantially from the ketamine effects just described. Most notably, peak norketamine concentrations of male rats stayed stable across age, although PD 40 and PD 80 female rats did have greater C_{max} values than males (see also Saland and Kabbaj, 2018). Unlike with ketamine, norketamine T_{max} varied greatly according to age and sex. In preweanling rats, norketamine T_{max} in the dorsal striatum was 75.9 min, which did not differ significantly from the T_{max} values of adult female rats ($\bar{x}$= 56.7 min), but was greater than the T_{max} values of adult male rats ($\bar{x}$= 11.2 min). The latter finding is consistent with previous studies showing that norketamine T_{max} in adult male rat brain occurs approximately 10 min after injections (Páleníček et al., 2011; Saland and Kabbaj, 2018). Norketamine AUC exhibited a ketamine-like pattern of effects, as female rats had AUC values that were constant across age, while male rats evidenced a general decline in AUC with increasing age. In sum, the various indices of norketamine pharmacokinetics did not vary according to age in female rats; however, male PD 40 and PD 80 rats had shorter latencies to reach peak norketamine levels, smaller peak values, and less total norketamine availability than female rats.

Curvilinear regression was used to more fully establish the relationship between age- and sex-dependent differences in ketamine/norketamine levels and locomotor activity. In both sexes and at all ages, the relationship between dorsal striatal ketamine concentrations and locomotion was best described by a cubic function in the general shape of an inverted U-shaped curve. In all male age groups and in younger female groups (PD 20 and PD 30) both high and low concentrations of dorsal striatal ketamine were associated with reduced locomotor activity, while moderate levels of ketamine correlated with increased locomotion. Presumably, the high concentrations of ketamine caused anesthesia and ataxia, while the low concentrations were insufficient to stimulate locomotion. In older female rats (PD 40 and PD 80), the correlations between brain ketamine levels and distance traveled scores were nonsignificant and the r^2 values small, suggesting that additional factors were influencing the ketamine-induced locomotor activity of these groups.

Dorsal striatal norketamine levels were also associated with locomotor activity and, once again, the relationship was strongest in male rats and young female rats (PD 20 and PD 30). In the case of norketamine, however, the relationship between these variables was best described by a linear or cubic function, in which the regression line increased across the scatterplot (male PD 80 rats were an exception). Thus, in most age groups, locomotor activity increased as brain norketamine increased. When the ketamine and norketamine results are considered together, it appears that ketamine is the critical component driving the anesthesia and ataxia evident at the start of the testing session. The basis for this conclusion is that high norketamine levels were associated with increased, rather than decreased, locomotion. Even so, the metabolite does have some anesthetic properties, since systemically administering a high dose of norketamine causes brief anesthesia followed by a marked increase in locomotor activity (Leung and Baillie, 1986). Consistent with the latter finding, rising concentrations of brain norketamine, as well as moderate concentrations of ketamine, were associated with increased locomotion in male rats and young female rats. It is curious that the locomotor activity of PD 40 and PD 80 female rats was not significantly related to brain ketamine or norketamine levels. Both of these groups showed high levels of locomotor activity towards the end of the testing session, when brain ketamine and norketamine levels were low, suggesting that additional ketamine metabolite(s) (e.g., hydroxyketamines, hydroxynorketamines, and dehydronorketamine) might be responsible for the late-stage locomotor activity exhibited by older female rats. (*2S,6S*)-Hydroxynorketamine is a possible candidate, since this metabolite produced a dose-dependent increase in the locomotor activity of adult male mice (Zanos et al., 2016).

Despite discrepancies involving adult female rats, it is clear that brain ketamine levels are tightly linked to locomotor activity in male rats and young female rats; however, the neural mechanisms by which ketamine increases locomotor activity remain uncertain. Initial studies found that ketamine increases DA release and/or blocks DA reuptake in the dorsal striatum and nucleus accumbens (Hancock and Stamford, 1999; Tso et al., 2004; Usun et al., 2013; Witkin et al., 2016), thus leading to the suggestion that ketamine may function like an indirect DA agonist at the presynaptic terminal (Hancock and Stamford, 1999; Irifune et al., 1991; Nishimura et al., 1998). Curiously, however, systemic ketamine administration has been reported to

increase (Irifune et al., 1991; Verma and Moghaddam, 1996; Witkin et al., 2016), decrease (Kari et al., 1978; Rao et al., 1989), or have no effect (Koshikawa et al., 1988; Verma and Moghaddam, 1996) on extracellular brain DA levels and/or DA turnover. Indeed, ketamine (80 mg/kg) did not alter DA levels in the dorsal striatum of male or female preweanling, adolescent, or adult rats when measured anywhere from 2.5 to 360 min after injection (see Fig. 2). Instead, ketamine may enhance locomotor activity by increasing the firing rate and burst firing of DA projection neurons in the ventral tegmental area (VTA) (see Belujon and Grace, 2014; French and Ceci, 1990; Witkin et al., 2016). NMDA receptors are expressed by tegmental DA neurons, as well as by cortical and subcortical neurons projecting to the VTA (Morikawa and Paladini, 2011). As a consequence of this neuroanatomical arrangement, there is ample opportunity for NMDA antagonists to modulate DA neurotransmission and locomotor activity.

In summary, ketamine and norketamine pharmacokinetics differs markedly depending on the age and sex of the rats being tested. In male rats (PD 20–PD 80) and young female rats (PD 20 and PD 30), moderate levels of dorsal striatal ketamine were associated with increased locomotor activity, while high concentrations of ketamine were associated with anesthesia and an absence of locomotion. Norketamine, on the other hand, may contribute to the locomotor response by stimulating locomotion in a concentration-dependent manner. The same relationships were not evident in older female rats (PD 40 and PD 80), suggesting that an additional factor, perhaps involving other ketamine and norketamine metabolites, was influencing the late-stage locomotor activity of these groups. More generally, the present results are consistent with various studies showing that low- and high-dose ketamine treatment causes profound age- and sex-dependent behavioral and neural effects that could have important translational relevance to humans (for reviews, see Wright and Kabbaj, 2018; Strong and Kabbaj, 2018).

Contributors

SAM was responsible for designing the study, statistical analyses, and drafting the manuscript. CAC conducted the ketamine and DA assays. GIP and GIR were involved in data collection and tissue preparation. VG and BCA were engaged in data collection. All authors contributed to the revising of the manuscript and approved the submission.

Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2019.03.013.

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