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Increased emotional reactivity in rats following exposure to caffeine during adolescence

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ABSTRACT

From 45 to 55 days after birth, male and female rats were treated via daily intraperitoneal injections with either isotonic saline, or 15 or 30 mg/kg caffeine. When 72–82 and 112–122 days old, their activity and emotional reactivity were assessed by means of frequencies of rearing, ambulation, immobility, defecation and urination recorded in an open field, as well as their occupancy of corners and center squares of the field, and their partial emergence and latencies to fully emerge from a small darkened chamber into a brightly lit arena. Rats treated with caffeine were probably more emotionally reactive than untreated controls as suggested by more immobility and defecation and urination. There were also effects on rearing and ambulation that might have arisen from increased impulsivity. Further evidence of caffeine treatment-induced higher emotional reactivity was found in the heavier adrenal glands of a small number of 10 months-old males. This occurred in the absence of any caffeine treatment effects on spatial reference memory measured by ability to identify a novel Y-maze arm. Changes between the two testing ages in rearing and emergence latencies, and sex-dependent changes in ambulation, defecation and corner and center squares occupancy, along with immobility for 30 mg/kg caffeine-treated subjects, were discussed in the light of possible changes in emotional reactivity. Sex differences in open-field rearing and ambulation, and testing age-dependent sex differences in corner and center squares occupancy were ascribed to higher emotional reactivity in males.

1. Introduction

Caffeine, the most popular drug on Earth [41], has been shown to affect the later behavior of rats and mice when exposed to the drug daily before birth [46,57] and during the lactational period of development either in their mothers' milk [14,34] or via subcutaneous injections [16]. A not infrequent result of gestational exposure to caffeine has been lower activity detectable soon after birth [14] and during adulthood for up to at least 6 months after treatment [34]. Similar outcomes have been observed following postnatal exposure during lactation [14,16,34,65] and during both gestation and lactation combined [14,34,49]. While male offspring are more susceptible than females to caffeine treatment during either gestation or lactation [32–34,36], this sex difference is not evident when exposed to the drug during both periods sequentially [17,35].

Decreased activity following gestational and/or lactational exposure to caffeine has been interpreted as a reflection of heightened emotional reactivity or timidity [32–34,36]. Evidence supporting this view includes increased open-field defecation [10,34], longer latencies to enter a conditioned aversive environment [55] or to emerge from a darkened chamber into a brightly lit arena [33,34], and greater preferences for a black rather than white environment [16]. In addition, perinatal caffeine has been associated with increased stress-related susceptibility to gastric ulcers [20] and higher adrenal weights [25], although this latter outcome may be more typical of male than female offspring [33,34,36].

While the evidence favoring increased emotional reactivity in laboratory rodents after perinatal exposure to caffeine seems reasonably convincing, the responsible mechanism has not yet been conclusively established. However, it is likely that adenosinergic neuromodulation is involved because, (1) caffeine's acute behavioral effects are most probably due to competitive antagonism of adenosine A_1 and A_{2A} receptors [19] and subsequent facilitation of neurotransmitter activity, especially dopamine [15] and acetylcholine [12], (2) chronic treatment with the drug can up-regulate A_1 receptors in the adult and newborn rat brain [42,53], and (3) rats exposed during both gestation and lactation to caffeine show heightened sensitivity to acute treatment with adenosine analogues [17,48]. Consequently, increased adenosinergic activity is a likely reason for higher perinatal caffeine-induced emotional reactivity that might be a reflection of greater behavioral inhibition and associated timidity [51].

Even though there are a number of reports describing subsequent effects of a range of drugs administered to rats and mice during their periadolescent stage of development, little is known about caffeine in this respect. This is in spite of research showing that a number of other drugs which are popular with human adolescents, such as alcohol, amphetamines and “party drugs”, can influence the course of later

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behavioral development when administered to adolescent rats [1,6,62]. In some cases, such treatment has resulted in the development of higher levels of emotional reactivity, as exemplified by later effects on adolescent rats of a common ingredient of party pills, 1-benzylpiperazine [1]. Many teenagers consume caffeine (especially in energy or soft drinks) on a daily basis [35]. Their consumption of the drug was estimated to be about 37 mg/day in one study [44] but for some individuals this can be as high as 800 mg/day [50] which is equivalent to about 6 or more 225-ml cups of brewed coffee [41]. Regular consumption of high levels of caffeine can lead to sleep difficulties and associated day-time tiredness in some adolescents [47] or even dependence [7] and increased health risks [52] if consumed in doses higher than 3 mg/kg/day [26]. In view of such outcomes and the fact that the adolescent brain is not fully mature either anatomically or neurochemically [59], it would not be surprising if chronic exposure to caffeine during this vulnerable period were to interfere with normal brain development. Therefore, in response to a call for more research to determine if long-term caffeine consumption by adolescents has adverse effects [26], the present study was designed to assess some subsequent behavioral effects of treating male and female rats with caffeine during the equivalent of late human adolescence, namely postnatal days 45 to 55 [62]. The doses chosen (15 and 30 mg/kg) were within the range previously shown to have subsequent behavioral effects on offspring when administered via daily intraperitoneal injections to pregnant rats [31]. The higher of these two doses is the maximum for stimulating motor activity in rats [45,58] without appearing to be unduly toxic or anxiogenic [13]. For a 70 kg human, 30 mg/kg would involve an intake of over 2 g caffeine (or more than 20 cups of coffee containing 100 mg caffeine/cup), a dose that is severely toxic and close to the lower limit of the estimated range of lethal doses [43]. However, if a correction is made for differences in metabolic rate between rats and humans [46], a dose of 30 mg/kg for a rat converts to a much safer dose of 8.8 mg/kg (or about 6 or 7 cups of coffee).

In view of increases in emotional reactivity following exposure to caffeine during gestation and/or lactation described above, particular attention was paid to such a possibility following exposure to the drug during adolescence. The persistence of any changes was assessed by recording emotionality-related behavior at two later ages, namely early and mid adulthood (72–82 and 112–122 days after birth). Although higher levels of emotional reactivity might be expected at the later testing age [8,40], earlier research has demonstrated increases with age in motor activities that are usually inversely related to emotionality [11,27,64].

At the completion of testing it was also decided to see if, at 10 months of age, there was any evidence of longer-lasting changes in spatial reference memory resulting from the rats' adolescent caffeine experience. This was because, although caffeine administered during the first week of life led to impaired learning of a spatial task involving working memory in adult rats [65], prenatal exposure to the drug enhanced retention of a passive avoidance response in female rats 25 days after training [60]. Acute administration of caffeine and other adenosine antagonists to adult rats and mice has been shown to enhance spatial learning and reference memory in a Morris water maze [2,61] and a radial maze [24] as well as improving performance on various memory tasks in humans, provided that the test conditions do not induce high arousal [56]. In the present study, effects of adolescent exposure to caffeine on spatial reference memory were assessed by the rats' unconditioned choices of novel stimuli that are guided by spatial cues [37] and known to be affected by memory-enhancing agents [28,29,38].

2. Methods

2.1. Subjects

The subjects were 27 male and 27 female PVG/C hooded rats chosen from 12 litters. All litters were of comparable size and contained approximately equal numbers of each sex. The rats were weaned at 30 days of age, caged in groups of 3–4 individuals of the same sex from different litters and kept in an ambient temperature of 22 °C±2 °C on a 12 h light/dark cycle (lights on at 08.00 h) with ad libitum food and water.

Procedures for housing, drug treatment and testing of all subjects complied with Parts 5 (Codes of Welfare) and 6 (Use of Animals in Research, Testing, and Teaching) of the New Zealand Animal Welfare Act (1999), and had been approved by the Animal Ethics Committee of the University of Canterbury.

2.2. Caffeine treatment

When 45 days old (P45), the 54 experimental subjects were randomly assigned to a control (0 mg/kg) group, a group treated with 15 mg/kg caffeine or a group treated with 30 mg/kg caffeine. These groups contained equal numbers of each sex and, as near as possible, equal numbers of rats from every litter. Each rat was then given a daily intraperitoneal injection (1 ml/kg) of its appropriate dose for 11 consecutive days. Control animals were administered isotonic saline, and caffeine-treated rats received caffeine dissolved in saline.

2.3. Behavioral testing

All rats experienced three open-field followed by three emergence tests between postnatal days 72 and 82 (early adulthood), and then again between postnatal days 112 and 122. There was an interval of three days between each pair of tests at each testing age. Exactly 40 days intervened between individual subject's set of tests at the younger and the older age. To minimize disruption caused by the rats being brought out of a darkened holding room into an illuminated research room, all testing occurred during the light phase of their light/dark cycle.

Approximately 3 mo later when the rats were between 8 and 9 mo old, their preference for a novel Y-maze arm was assessed to determine whether or not their caffeine treatment during adolescence had affected their ability to remember which of the arms was previously inaccessible. This was followed a month later by a single emergence test for 8 control (4 males, 4 females) and 8 rats (4 males, 4 females) that had been treated with 30 mg/kg caffeine during adolescence. These rats were randomly selected from the two treatment groups. After testing, they were sacrificed, weighed and their left adrenal gland removed, cleaned of surrounding tissue and its weight determined relative to the rat's body weight (mg/100 g). The small numbers of subjects used for this phase of the study arose from the need to cull the remainder because of animal housing requirements.

2.3.1. Open-field tests

Through observations of defecation, urination, ambulation and other forms of activity, open-field tests have been a popular way of assessing emotional reactivity in rats for over 70 years [9,22,63]. In general, low activity, frequent defecation, urination and freezing, low occupancy of the center of the apparatus and high occupancy of the corners of a square field are regarded as indicative of high emotional reactivity [3,5,9,22]. The apparatus used in the present study was a 600×600 mm wooden open field with walls 250 mm high. It was painted black and the floor was divided into 16 squares by a grid of intersecting white lines. The open field sat on a 700-mm high table and was illuminated by dim (47 lx) overhead fluorescent lighting. An infrared video camera was mounted 850 mm above the floor of the apparatus, and all behavior of each individual rat was video-recorded for 5 min after having been placed in the center of the open field. The rat was then removed and the number of fecal boluses it left in the apparatus (defecation) and the number of times it had urinated (urination) were counted before the field was washed with a 2%

Table 1
Mean (±S.E.M.) values of each open-field and emergence test measure (except urination) for both sexes and testing ages combined following adolescent caffeine treatment, and results of ANOVAs

	Caffeine treatment dose (mg/kg)				
	0 (n=16)	15 (n=18)	30 (n=18)	F(2, 46)	p
Rearing	34.50 (±2.73)[a]	28.47 (±1.86)[a,b]	35.34 (±1.68)[b]	3.40	.042
Ambulation	73.76 (±5.04)	64.79 (±4.67)[a]	79.32 (±5.78)[a]	3.74	.031
Immobility*	0.96 (±0.12)[a]	1.46 (±0.12)[b]	2.08 (±0.43)[a,b]	10.74	<.0001
Corner occupancy	50.34 (±1.75)	54.32 (±2.62)	53.24 (±1.78)	0.76	>.4
Center occupancy	5.25 (±0.34)	3.89 (±0.51)	5.07 (±0.56)	2.30	>.1
Defecation	0.51 (±0.16)[a]	1.07 (±0.37)	1.87 (±0.43)[a]	3.45	.04
Emergence (s)	110.31 (±18.84)	143.03 (±18.06)[a]	66.87 (±16.51)[a]	4.47	.017
Head pokes	4.40 (±0.51)[a]	6.41 (±0.68)[b]	3.44 (±0.41)[a,b]	7.51	.002

[a,b]Difference between the two groups with superscripts in common significant, $p<.05$, Neumann–Keuls test. *Caffeine treatment×testing age interaction significant (see text).

solution of Powerquat Blue disinfectant. The video tapes were later viewed and the following forms of behavior recorded for each rat:

(1) the number of times it reared up on its hind legs (rearing)
(2) the number of lines crossed by its hind legs (ambulation)
(3) the number of times it remained completely immobile for more than 3 s (immobility)
(4) the number of 3-s observations (signaled by an auditory timer and earphone) in which it was occupying one of the four corners (corner occupancy) or four center squares of the apparatus (center occupancy).

2.3.2. Emergence tests

After completion of an open-field test, each rat was returned to its home cage for several minutes and was then placed in a small darkened chamber in order to measure its speed of emergence into a larger brightly lit arena. The longer it takes for a rat to emerge into a novel area is generally accepted as reflecting higher levels of emotional reactivity, fear or timidity [3]. The apparatus comprised a 200×150×200-mm-high black-painted wooden box that opened by means of a sliding door into a 500×400×200-mm-high arena with a translucent Perspex floor that was illuminated from underneath by two 16-w fluorescent tubes. The light level in the arena was 172 lx, and it was covered by a wire-mesh lid. The apparatus sat on a 700-mm-high table in the same room as the open field.

An emergence test consisted of placing a rat in the darkened chamber and, approximately 10 s later, opening the sliding door to allow it access to the brightly lit arena. The number of times it partially emerged was counted (head pokes), and the time it took to fully emerge was recorded by a hand-held stop watch. If it had not fully emerged after 5 min, the trial was terminated and an emergence latency of 300 s recorded.

2.3.3. Preferences for a novel Y-maze arm

As with other measures of preferences for novel locations, this test exploits rats' natural curiosity and draws upon memory, fear and the rewarding properties of novelty [30]. Because identification of novel stimuli in a Y-maze involves the use of spatial cues within and outside of the apparatus, the test is primarily one of spatial memory [37]. In the present study, the procedure was designed to maximally involve reference memory.

The apparatus consisted of a one of four identical wooden Y-mazes that sat on 700-mm-high tables beneath dim room fluorescent lighting. The arms of each maze were 45 cm long with an angle of 120° between them, and the stem was 15 cm long. They each contained a black painted aluminum insert that covered the floor, side walls and end wall, and entry to one of the arms could be prevented by a wooden guillotine slide placed across its entrance. All parts of the maze were 10 cm wide, 14 cm high and were covered by transparent Perspex lids.

The testing procedure involved confining individual rats to the apparatus with one of the arms inaccessible for a 2-h acquisition trial. It was then returned to its home cage and, 24 h later, placed in the stem of the same Y-maze with the slide blocking entries into one of the arms removed, and continuously observed for a 3-min retention trial. By means of a computer keyboard and specially written program, the number of entries of and time spent in each arm were recorded. Two days later, the test was repeated with the opposite arm blocked to that which was blocked for the first acquisition trial. Thus, for each rat, the previously blocked novel arm was on the left for one test, and on the right for the other.

3. Results

Unfortunately two male control rats died in between the first and second series of open-field and emergence testing at postnatal days 72–82 and 112–122. Therefore, treatment (3)×sex (2)×testing age (2) ANOVAs were performed on all measures recorded in the open field (except urination) and emergence apparatus for the remaining 52 rats that completed testing at both ages. As it was clear that there were no consistent patterns of change for any group on any measure between the three tests conducted at each testing age, the ANOVAs were carried out on individual rats' averages of the three. When significant caffeine treatment effects occurred, post hoc comparisons were made between all individual groups by means of Neumann–Keuls tests. Because of large numbers of 0 scores and thus highly skewed distributions, urination was subjected to nonparametric median tests [54] to assess the effects of adolescent caffeine treatment and sex on numbers of rats that were not seen to ever urinate. An overall comparison of urination at the two testing ages was made by means of a sign test [54].

First entries of and percentages of subsequent entries of and time spent in the novel versus familiar arm of a Y-maze, and total entries of and time spent in both arms when the rats were 8–9 mo old were

Table 2
Mean (±S.E.M.) values of each open-field and emergence test measure (except urination) for males (n=25) and females (n=27) and for postnatal days 72–82 and 112–122 (adolescent caffeine treatment groups combined), and results of ANOVAs

	Sex				Postnatal days			
	Males	Females	F(2, 46)	p	72–82	112–122	F(1, 46)	p
Rearing	29.31 (±1.69)	36.85 (±1.66)	8.64	.005	31.05 (±1.40)	35.11 (±1.50)	4.39	.042
Ambulation*	57.08 (±3.24)	86.93 (±3.17)	47.22	<.0001	72.69 (±2.95)	71.32 (±3.46)	0.18	>.6
Immobility**	1.68 (±0.19)	1.38 (±0.14)	2.07	>.1	1.83 (±0.17)	1.23 (±0.11)	17.72	<.0001
Corner occupancy*	54.65 (±1.95)	50.93 (±1.43)	2.21	>.1	54.33 (±1.18)	51.25 (±1.57)	5.76	.021
Center occupancy*	4.30 (±0.40)	5.10 (±0.41)	1.79	>.1	4.58 (±0.31)	4.82 (±0.38)	0.34	>.5
Defecation*	1.53 (±0.31)	0.85 (±0.29)	2.37	>.1	1.39 (±0.26)	0.99 (±0.21)	4.88	.032
Emergence (s)	120.24 (±18.02)	93.96 (±12.92)	1.64	>.2	96.24 (±11.94)	117.96 (±11.87)	4.59	.038
Head pokes	4.84 (±0.46)	4.69 (±0.53)	0.05	>.8	4.44 (±0.36)	5.09 (±0.44)	2.66	>.1

*Sex×testing age interaction significant (see text). **Caffeine treatment×testing age interaction significant (see text).

Table 3
Mean (±S.E.M.) values of four open-field measures for males (n=25) and females (n=27) recorded at postnatal days 72–82 and 112–122 (adolescent caffeine treatment groups combined) for which sex×testing age interactions were significant

	Males		Females	
	Postnatal days 72–82	Postnatal days 112–122	Postnatal days 72–82	Postnatal days 112–122
Ambulation	59.27 (±3.36)[a,c]	54.89 (±3.70)[b,c]	85.11 (±3.30)[a,d]	88.74 (±3.51)[b,d]
Corner occupancy	54.09 (±1.62)[e,g]	55.21 (±2.67)[f,g]	54.06 (±1.75)[e,h]	47.31 (±1.43)[f,h]
Center occupancy	4.63 (±0.39)[i,k]	3.97 (±0.52)[j,k]	4.54 (±0.48)[i,l]	5.65 (±0.51)[j,l]
Defecation	2.04 (±0.40)[m,o]	1.01 (±0.31)[n,o]	0.79 (±0.31)[m,p]	0.91 (±0.30)[n,p]

[a–p]Values of F(1,46) and probability levels for comparisons between groups with superscripts in common, [a]32.12, $p<.0001$, [b]48.92, $p<.0001$, [c]4.05, $p=.05$, [d]2.16, $p>.1$, [e]0.04, $p>.8$, [f]6.46, $p=.014$, [g]0.32, $p>.5$, [h]16.49, $p<.0001$, [i]0.06, $p>.8$, [j]4.91, $p=.032$, [k]1.49, $p>.2$, [l]4.46, $p=.04$, [m]6.06, $p=0.018$, [n]0.03, $p>.8$, [o]12.36, $p<.001$, [p]0.21, $p>.6$.

subjected to treatment (3)×sex (2) ANOVAs. In view of sex-related differences in body and relative adrenal weights following exposure to caffeine during gestation [33] and during gestation and lactation sequentially [36], differences between the 0 and 30 mg/kg caffeine-treated rats were assessed by separate t-tests. A similar approach also typified emergence latencies that had been recorded prior to the determination of adrenal weights.

3.1. Open-field and emergence tests at postnatal days 72–82 and 112–122

3.1.1. Adolescent caffeine treatment effects

Mean±S.E.M scores following adolescent caffeine treatment for all measures recorded in the open field (except urination) and emergence apparatus during postnatal days 72–82 and 112–122 combined can be seen in Table 1.

Significant caffeine treatment effects occurred for all measures except occupancy of corners or center squares of the open field. Rats that had been treated with 15 mg/kg caffeine exhibited less rearing than those in either other group and less ambulation than rats treated with 30 mg/kg caffeine. These latter rats were also more immobile than those in the other groups, defecated more than control animals, emerged faster from the darkened chamber than rats treated with 15 mg/kg caffeine and made fewer head pokes than those in either other group. (A significant interaction occurred between adolescent caffeine treatment and testing age for immobility, F(2, 46)=4.30, $p=.019$, and will be described in the next section (3.1.2) dealing with sex and testing age differences.).

The numbers of rats that did not urinate in the open field on any of their six opportunities following adolescent treatment with 0 (n=16), 15 (n=18) and 30 mg/kg (n=18) caffeine respectively were 5 (31.25%), 3 (16.67%) and 4 (22.22%). Differences between these groups were not significant, $\chi^2(2)=3.20$, $p>.2$.

3.1.2. Differences between the two sexes and testing ages

As can be seen in Table 2, female rats showed significantly higher overall frequencies of rearing and ambulation in the open field than males. More rearing accompanied by less immobility, corner occupancy and defecation, and longer emergence latencies occurred at the older testing age than when the rats were younger.

However, there were also significant interactions between sex and testing age in ambulation, F(1,46)=6.06, $p=.018$, corner occupancy, F(1,46)=10.32, $p=.002$, center occupancy, F(1,46)=5.46, $p=.024$, and defecation, F(1,46)=8.06, $p=.007$, along with the significant caffeine×testing age interaction for immobility referred to in the previous section. The sex×testing age interactions are outlined in Table 3.

These revealed significantly more ambulation for females than for males at both testing ages, and a significant decrease in the response from postnatal days 72–82 to postnatal days 112–122 for males but not for females. While females occupied open-field corners less and center squares more than males at the older testing age, the two sexes did not significantly differ on these measures at the younger age. Females also occupied corners less and center squares more at the older than at the younger age, but this was not so for males. Males defecated more than females at the younger but not the older testing age, and, contrary to females, defecated less at the older than at the younger age.

The caffeine×testing age interaction for immobility arose from, a significant decrease between days 72–82 and 112–122 only for rats treated with 30 mg/kg caffeine i.e., 2.69 (±0.33) and 1.48 (±0.19) respectively. There were no significant changes between the two testing ages for either the control, F(1,46)=2.44, $p>.1$, or 15 mg/kg caffeine-treated groups, F(1,46)=0.01, $p>.9$, and the caffeine treatment effect remained significant at testing ages 72–82, F(2,46)=10.84, $p<.001$, and 112–122, F(2,46)=4.17, $p=.022$.

Numbers of males (n=25) and females (n=27) that did not urinate in the open field on any occasion were 4 (16.00%) and 8 (29.63%) respectively. This sex difference was not significant, $\chi^2(1)=1.36$, $p>.2$. For both sexes combined, 13 rats urinated less, 17 urinated more and 22 showed no change between postnatal days 72–82 and 112–122. These numbers did not differ significantly, $z=0.55$, $p>.5$.

3.2. Preferences for a novel Y-maze arm at 8–9 months of age

Results for the measures of novelty preference, namely first and subsequent entries of and time spent in the novel arm, and also total entries of and time spent in both arms when the rats were 8–9 months old are outlined in Table 4.

Neither adolescent caffeine treatment nor sex affected their ability to identify and prefer the novel arm as determined by first and subsequent entries of and time spent in it. However, all rats combined chose to re-enter and spend time in this arm significantly more often than expected by chance, namely, percent entries, mean (±S.E.M)=53.39 (±0.72), one-sample t(51)=4.56, $p<.0001$; percent time=56.17 (±1.27), t(51)=4.85, $p<.0001$. Caffeine treatment did not affect either the number of times both arms were entered or the time spent in them, but in both cases females achieved significantly higher scores than males.

Table 4
Mean (±S.E.M.) values of first entries of, percent entries of and percent time spent in the novel arm of a Y-maze, total entries of and time spent in both arms recorded at 8–9 months of age following adolescent caffeine treatment, and results of ANOVAs

	Caffeine treatment dose (mg/kg)					Sex			
	0 (n=16)	15 (n=18)	30 (n=18)	F(2, 46)	P	Males (n=25)	Females (n=27)	F(1, 46)	p
First entries	1.06 (±0.11)	1.33 (±0.16)	1.06 (±0.15)	1.17	>.3	1.24 (±0.13)	1.07 (±0.11)	0.89	>.3
% Novel entries	55.06 (±0.31)	52.05 (±1.26)	52.95 (±1.22)	1.55	>.2	53.15 (±1.03)	53.42 (±1.03)	0.01	>.9
% Novel time	57.76 (±2.02)	53.32 (±2.22)	57.60 (±2.28)	1.25	>.2	55.24 (±2.07)	57.03 (±1.55)	0.52	>.4
Total entries	14.29 (±0.63)	13.11 (±0.64)	13.41 (±0.65)	0.89	>.4	12.15 (±0.48)	14.89 (±0.42)	16.69	<.0005
Total time (s)	156.57 (±10.71)	140.16 (±8.87)	151.02 (±8.24)	0.81	>.4	127.48 (±6.28)	168.87 (±6.42)	20.39	<.0002

3.3. Emergence test and relative adrenal weights at 10 months of age

Emergence latencies for 10-months-old male rats that had been exposed to either 0 or 30 mg/kg caffeine during adolescence were 261.50 (±37.18) and 148.50 (±58.66) respectively. Comparable latencies for females were 122.25 (±37.97) and 115.25 (±60.54). The difference between the treatment conditions was not significant for either males, $t(6)=1.63$, $p>.1$, or females, $t(6)=1.52$, $p>.1$. However, overall females, mean±S.E.M.=68.75 (±33.28), emerged significantly faster than males, 205.00 (±38.59), $t(14)=2.67$, $p=.018$. Although females' body weights, 212.16 (±7.49) g, were significantly lighter overall than those of males, 371.9 (±14.85), $t(14)=9.60$, $p<.0001$, the weights for neither sex were affected by their adolescent caffeine experience i.e., males 0 mg/kg=354.43 (±21.46), 30 mg/kg=389.38 (±19.11), $t(6)=1.22$, $p>.1$; females 0 mg/kg=200.38 (±3.66), 30 mg/kg=223.95 (±12.49), $t(6)=1.81$, $p>.1$. Females' relative adrenal weights (mg/100 g), 14.11 (±0.77), were significantly heavier overall than those of males, 7.30 (±0.64), $t(14)=6.78$, $p<.0001$. However, as outlined in Fig. 1, adolescent caffeine treatment increased the relative weights for males, $t(6)=2.41$, $p=.052$, but not for females, $t(6)=1.52$, $p>.1$.

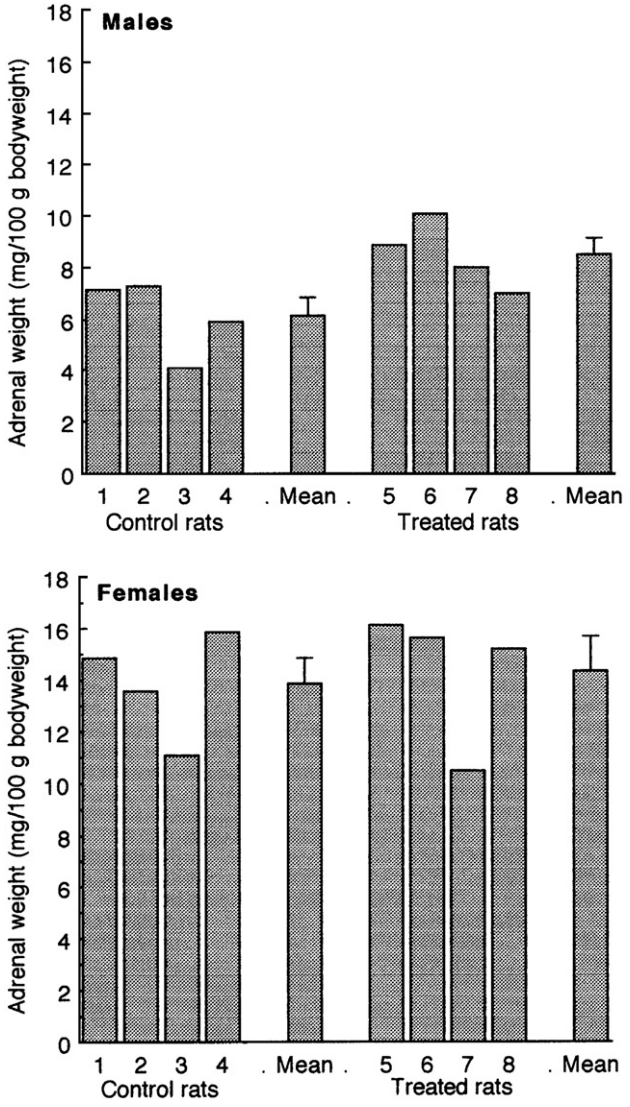

Fig. 1. Relative adrenal weights for 10-months-old individual male and female rats and mean (±S.E.M.) weights for each sex following treatment with saline or 30 mg/kg caffeine during adolescence.

4. Discussion

Clearly, exposure of the rats to caffeine during adolescence resulted in a number of significant outcomes that would at least justify further research and perhaps question the wisdom of consumption of high doses of caffeine by human adolescents. This is because treatment with the drug increased immobility and defecation in the open field thereby suggesting that it had produced small but long-lasting increases in emotional reactivity [2]. This was supported by a similar outcome for 10-months-old male (but not female) rats exposed to 30 mg/kg caffeine to what has been repeatedly described for gestational and/or lactational exposure to caffeine [33,34,36], namely increased relative adrenal weights. Although a rather crude measure of sympathetic responsiveness, higher weights can indicate the effects of a chronically stressful condition [23,39]. The lack of any effect of caffeine on relative adrenal weights in females may have arisen from a ceiling effect caused by the adrenal weights of untreated subjects being significantly heavier than those of males (see Fig. 1). However, it is possible that the significant effect for males only was merely an aberration due to small sample sizes, although the sex-related outcome was consistent with effects of perinatal caffeine treatment [33,34,36].

The caffeine treatment also had some complicated effects on ambulation and rearing in the open field i.e., the lowest frequencies of each were associated with exposure to 15 mg/kg while there was no significant difference between 0 and 30 mg/kg. Since both measures are believed to be negatively related to emotionality [3], this would suggest that 15 mg/kg had increased emotional reactivity but that 30 mg/kg was paradoxically ineffective in this respect. However, it is possible that while the lower of the two caffeine doses may have indeed increased emotional reactivity and thus interfered with any curiosity-related basis for the two responses, the higher dose may have similarly increased emotional reactivity and suppressed curiosity but may also have initiated fear-induced attempts to escape from the apparatus. This was supported in particular for rearing activity by casual observations made by NLA who noted that this response seemed to reflect either a more relaxed curiosity-related “interest” in the upper parts of the apparatus, or rather “frenetic” behavior that gave the appearance of attempts to escape. Obviously further research is required to establish whether or not it is possible to distinguish between two types of ambulation and rearing in terms of their specific motivational substrates.

The finding that the shortest emergence latencies in the emergence apparatus occurred with rats exposed to 30 mg/kg caffeine would also appear to be contrary to the possibility that the drug had increased emotional reactivity. On the other hand, the fact that the lowest frequencies of partial emergence in the form of head pokes also occurred with this group, might be interpreted as being due to increased emotional reactivity. However, this seems improbable because of a positive Pearson correlation between the two responses for all rats combined i.e., $r=0.76$, $p(50)<.001$. Instead, as supported by casual observations, it seems more likely that both responses reflected an increase in impulsivity that has been shown to follow acute caffeine administration in rats [18].

The failure for adolescent caffeine treatment to have any effect on the rats' ability to select the more novel of two Y-maze arms when they were 8–9 months old suggests that their experience had neither impaired nor enhanced retention of the task. In view of caffeine's acute effects on performance in spatial tasks [2,24,61] and impaired learning ability following exposure to the drug prenatally in mice [55] or during the first week of life in rats [65], this outcome was surprising. However, whether or not caffeine would have affected responses to the novel Y-maze arm in the present study if the animals had been tested at younger ages remains to be established. It is also possible that the task was not sufficiently demanding on spatial reference memory for any treatment effects to be noticeable and, in

future work, should perhaps be replaced with tests (such as the Morris water maze) that more specifically target spatial ability and also enable distinctions to be drawn between the operation of working and reference memory.

Sex differences favoring females in open-field rearing and ambulation for postnatal days 72–82 and 112–122 combined, and entries of and time spent in both Y-maze arms at 8–9 months of age were consistent with the view that females are more active than males [3]. There were also some other sex differences that were dependant on the age at which the rats were tested namely, less corner and more center squares occupancy for females than for males when the rats were tested at postnatal days 112–122, but no sex differences in these measures at postnatal days 72–82. On the other hand, males defecated more than females when tested at the younger age, but not when older. These sex difference were consistent with the view that male rats are more emotionally reactive than females [4,21].

Increases in rearing and emergence latencies for all rats between the two testing ages suggests that, in line with earlier conclusions [8,40], they may have become more emotionally reactive as they grew older. This is because they were slower to emerge from the darkened chamber of the emergence apparatus and engaged in more possibly escape-related rearing at the later testing age. This is supported by emergence latencies when the rats were 10 months old which were noticeably longer than those recorded at either of the earlier ages. However, a contrary interpretation would follow the observations that, for males only, ambulation and defecation declined between the two ages, whereas corner occupancy declined and center squares occupancy increased for females alone. In addition, immobility also declined only for rats that had been treated with 30 mg/kg caffeine. So, while some of the changes suggest an increase in emotional reactivity with age, namely rearing (possibly) and emergence latencies, others suggest a decrease as reported earlier [11,27,64] i.e., sex-dependent ambulation (possibly), corner and center squares occupancy, defecation and caffeine treatment-related immobility! Clearly, the behavioral processes underlying these different age-related changes can not be conclusively identified without further research.

The effects of treatment with caffeine during adolescence on later immobility, defecation, relative adrenal weights and maybe rearing and ambulation suggest heightened emotional reactivity in a similar manner to that concluded for the subsequent effects of perinatal exposure to the drug [33,34]. It is possible that caffeine-treated rats' adolescent experience also increased impulsivity [18], as suggested by their emergence latencies and number of head pokes in the emergence apparatus. Overall, it seems likely that the results of the study were due to caffeine effects on adolescent brain development possibly involving adenosine-facilitated increases in neurotransmitter activity [19], especially dopamine [15], comparable to what probably characterises pre- and early postnatal development. In addition to having possible implications for the risks of caffeine consumption by human adolescents, the results highlight the need in future research to determine how critical adolescence really is in this respect, vis-à-vis other ages that are not commonly regarded as important identifiable stages of brain development.

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