

## Dimorphism and structural modulation in quinoxaline

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**The  $Z' = 1$  and  $Z' = 5$  structures of quinoxaline are compared. The nature of the intermolecular interactions in the  $Z' = 5$  structure is studied by means of variable-temperature single-crystal X-ray diffraction. The C–H...N and  $\pi \cdots \pi$  interactions in these structures are of a stabilizing nature. The high  $Z'$  structure has the better interactions, whereas the low  $Z'$  structure has the better stability. This trade-off is a recurrent theme in molecular crystals and is a manifestation of the distinction between thermodynamically and kinetically favoured crystal forms.**

**Keywords:** Crystal structure and engineering, cryocrystallization, hydrogen bond, polymorphism.

THE number of molecules in the asymmetric unit,  $Z'$ , is a property of fundamental significance in a molecular crystal. Unless the molecule occupies a crystal symmetry site,  $Z'$  is generally equal to one. Crystal structures with  $Z' > 1$  are of interest. Why are such structures even observed<sup>1–3</sup>? It is difficult to rationalize their occurrence in each and every case. Conformational flexibility, awkward molecular shape, molecular chirality, solvent effects and in many cases, conflicts between molecular packing and intermolecular interactions are some of the common arguments put forth for the occurrence of high  $Z'$  structures. The high  $Z'$  phenomenon has also been linked to the crystallization mechanism. It is proposed that some molecules exist as structured aggregates in the pre-crystallization stages. It is possible that these aggregates retain their identity as high  $Z'$  growth units; they would be kinetically preferred over other slower evolving low  $Z'$  motifs. Experimental isolation of both high and low  $Z'$  polymorphs of the same compound is useful because it permits a more detailed analysis of the phenomenon in terms of interactions and stabilities. In cases where the high  $Z'$  structure is less stable than the low  $Z'$  form, it is possible that the former is a crystal 'on the way'.

The crystal structure of quinoxaline, 1,4-diazanaphthalene,  $C_{10}H_6N_2$ , in the space group  $P2_12_12_1$  ( $Z' = 5$ ), CSD refcode HEYJOK, was reported more than a decade ago<sup>4</sup>. Quinoxaline is a rigid and planar molecule with no strong hydrogen-bond donors. Why does this molecule take a high  $Z'$  structure, and that too with  $Z' = 5$ ? The environ-

ment around each of the five symmetry-independent molecules in this structure is also largely the same and the possibility of modulation was alluded to in the previous study. To explore this possibility further, we have reinvestigated this substance and have recently reported a new  $Z' = 1$  form of quinoxaline that is structurally related to the previously reported  $Z' = 5$  structure<sup>5</sup>. In this communication, we compare the two forms in the context of the crystallization mechanism.

The analysis takes the form of determining the crystal structure of the  $Z' = 5$  structure at several temperatures, and comparing the geometrical parameters in these structure determinations with those in the  $Z' = 1$  form. Quinoxaline melts at 29–32°C. Good quality crystals of both the  $Z' = 1$  and  $Z' = 5$  forms were grown *in situ* by means of cryocrystallization techniques that have been described elsewhere<sup>6</sup>. Concomitancy is common in this system. Data were collected for both forms initially at 270 K and then for the  $Z' = 5$  crystal at several other lower temperatures (Table 1). Comparison of the unit cell parameters suggests that modulation is present (270 K, Table 1). ORTEP diagrams for the two structures at 270 K are shown in Figure 1 for comparison. The two structures show minor structural differences as suggested by the low rmsd value of 0.2580 Å (calculated for the non-hydrogen atoms using the coset software<sup>7</sup>, by taking 50 molecules and a distance tolerance of 5%). Symmetry-independent molecules in the  $Z' = 5$  structure differ in terms of their relative orientations leading to some asymmetry in the crystal structure. A packing overlay of the  $Z' = 1$  and  $Z' = 5$  unit cells establishes a pseudo-translation relationship between the symmetry-independent molecules in the  $Z' = 5$  structure (Figure 2). Molecules in the  $Z' = 1$  structure are held by weak intermolecular contacts of the C–H...N and  $\pi \cdots \pi$  type and are shown in Figure 3. The C–H...N interaction in the  $Z' = 1$  structure (3.555(3) Å) is longer than any of the five distances in the  $Z' = 5$  structure (3.418(4)–3.541(4) Å; average value 3.511 Å). The details are given in Table 2. A similar behaviour is observed for the  $\pi \cdots \pi$  interactions in the two polymorphs (Table 3).

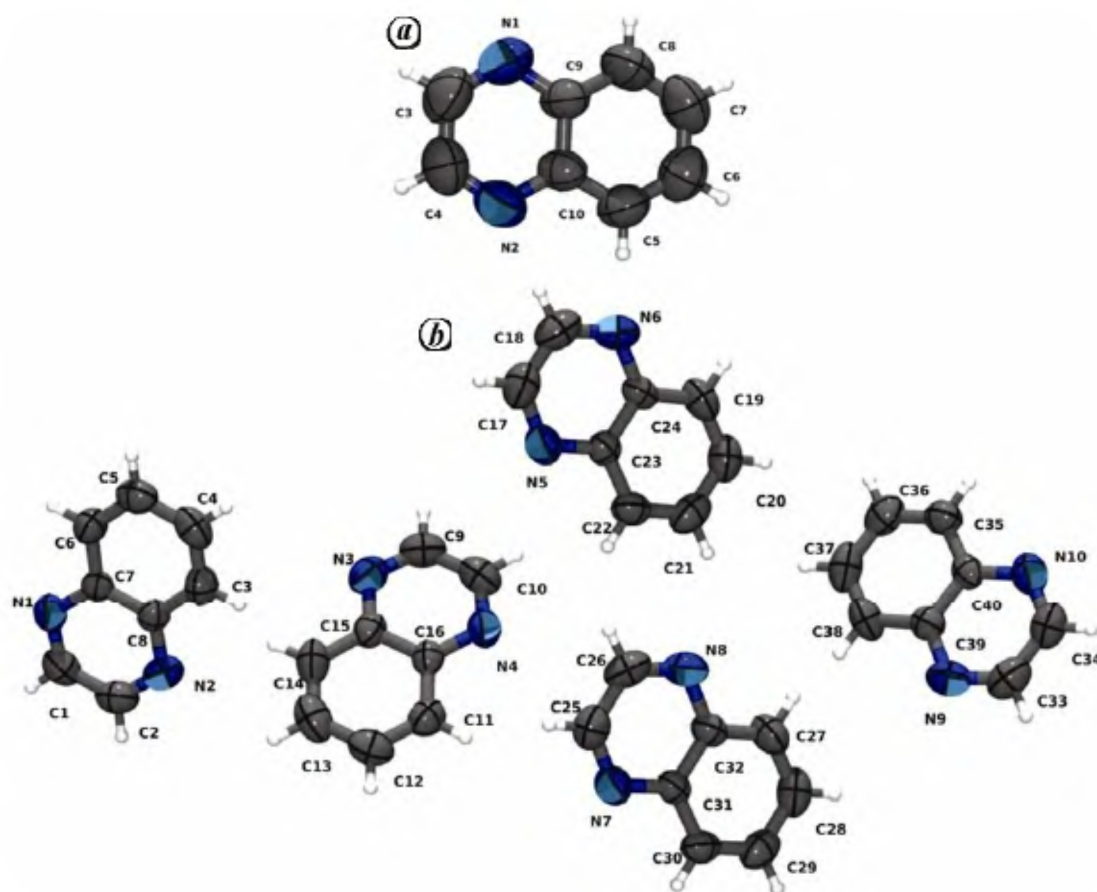
The nature of the C–H...N contacts in the  $Z' = 5$  structure was examined by means of a variable temperature X-ray diffraction study. We have shown earlier that true hydrogen bonds are shortened and straightened out upon cooling<sup>8</sup>. Shortening of the C...N distance on lowering of temperature (Figure 4) clearly establishes the hydrogen bond character for these C–H...N interactions. Comparison of the relative densities, 1.295 and 1.311 g cm<sup>–3</sup>, and the Kitaigorodskii packing index<sup>9</sup>, 67.2% and 68.4% respectively, for the  $Z' = 1$  and  $Z' = 5$  structures, indicates a slightly better packing for the  $Z' = 5$  structure.

Lattice energy calculations<sup>10</sup> were performed with the Dreiding force field. These show that the  $Z' = 1$  structure is more stable than the  $Z' = 5$  structure by 0.78 kcal/mol (Table 4). Comparison of the electrostatic and van der

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**Table 1.** Crystallographic data and structure refinement parameters for the  $Z' = 1$  and  $Z' = 5$  structures of quinoxaline. (Structural formula:  $C_8H_6N_2$ ; Formula weight: 130.15; Crystal system: orthorhombic; Space group:  $P2_12_12_1$ .)

Temperature (K)	90(2)	110(2)	130(2)	150(2)	170(2)	190(2)	270(2)	270(2)
$Z$	20	20	20	20	20	20	20	4
$a$ (Å)	3.8917(9)	3.9157(11)	3.9241(8)	3.9330(9)	3.9426(8)	3.9602(8)	3.996(2)	4.0212(13)
$b$ (Å)	22.900(5)	22.983(6)	22.983(5)	22.988(5)	22.987(5)	23.023(5)	23.022(12)	7.187(2)
$c$ (Å)	35.252(8)	35.391(10)	35.412(7)	35.438(8)	35.459(7)	35.542(7)	35.822(19)	23.095(3)
$V$ (Å <sup>3</sup> )	3141.7(12)	3185.0(15)	3193.7(11)	3204.0(12)	3213.6(11)	3240.6(12)	3296(3)	667.5(3)
$\rho_{\text{calc}}$ (g/cm <sup>3</sup> )	1.376	1.357	1.353	1.349	1.345	1.334	1.311	1.295
$\mu$ (mm <sup>-1</sup> )	0.086	0.085	0.085	0.084	0.084	0.083	0.082	0.081
$R_1$ [ $I > 2\sigma(I)$ ]	0.0601	0.0726	0.0589	0.0597	0.0589	0.0614	0.0415	0.0377
$wR_2$	0.1784	0.2290	0.1737	0.1722	0.1657	0.1764	0.1007	0.1060
$\theta_{\text{min}}-\theta_{\text{max}}$ (°)	1.8–27.8	1.8–28.1	1.5–28.0	1.8–27.9	1.5–28.0	1.5–28.0	1.4–28.4	1.8–27.79
Goodness-of-fit	1.09	1.09	1.11	1.04	1.05	1.05	0.78	0.94
Reflections collected	34807	34819	35710	35876	35978	36086	36700	7556
Unique reflections	4319	4479	4480	4495	4496	4547	4554	956
Observed reflections	2407	2411	2486	2419	2356	2143	1739	494
CCDC no.	792115	792116	792117	792118	792119	792120	792121	Ref. 7

**Figure 1.** ORTEP drawn at 50% probability level for non-H atoms for quinoxaline (a)  $Z' = 1$  and (b)  $Z' = 5$  structure.

Waals interaction terms in the two cases shows slightly higher (more negative) contribution from the electrostatic energy terms for the  $Z' = 5$  structure. This can be related to the shorter C–H...N interactions observed in that structure. However, the contribution from the van der Waals term for the  $Z' = 5$  structure was found to be less favour-

able than in the  $Z' = 1$  structure and this was also found to worsen further with a decrease in temperature. Higher density and better packing are generally considered to be signatures of the thermodynamically most stable form. However, in the present case the less stable  $Z' = 5$  structure showed slightly better packing and better interactions

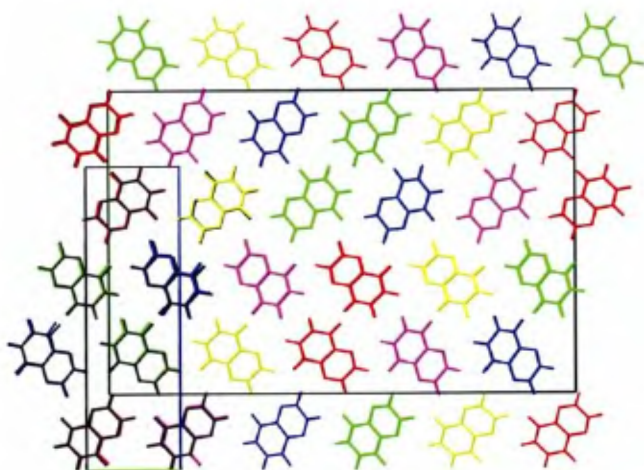
**Table 2.** Variation in C...N distance (Å) for C–H...N interactions in the  $Z' = 5$  form of quinoxaline as a function of temperature. The distance for the  $Z' = 1$  form at 270 K is given for reference

Structure	Code	Contact	90 K	110 K	130 K	150 K	170 K	190 K	270 K
$Z' = 5$	A	C1–H1...N10	3.454(6)	3.472(7)	3.477(6)	3.473(6)	3.487(5)	3.501(6)	3.541(4)
	B	C10–H10...N5	3.410(6)	3.429(8)	3.429(6)	3.442(6)	3.444(5)	3.454(6)	3.493(4)
	C	C17–H17...N1	3.412(6)	3.433(8)	3.431(6)	3.445(6)	3.444(5)	3.463(6)	3.494(4)
	D	C25–H25...N4	3.428(6)	3.438(7)	3.447(6)	3.463(6)	3.465(5)	3.482(6)	3.547(4)
	E	C34–H34...N7	3.387(6)	3.408(8)	3.410(6)	3.421(6)	3.425(5)	3.442(6)	3.481(4)
		Average	3.418	3.436	3.439	3.449	3.453	3.468	3.511
$Z' = 1$		C4–H4...N2	–	–	–	–	–		3.555(3)

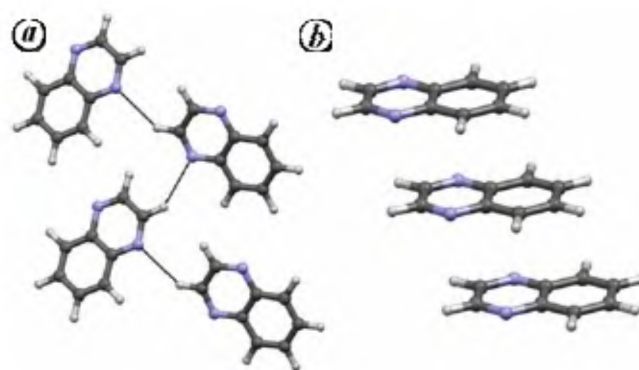
**Table 3.** Variation in the interplanar distances (Å) for the  $\pi \cdots \pi$  stacking interaction in the  $Z' = 5$  form of quinoxaline as a function of temperature. The value for the  $Z' = 1$  form at 270 K is given for reference

Structure	Code	Contact*	90 K	110 K	130 K	150 K	170 K	190 K	270 K
$Z' = 5$	1	Cg(1)...Cg(2)	3.459(2)	3.479(2)	3.503(2)	3.509(2)	3.516(2)	3.533(2)	3.563(1)
	2	Cg(4)...Cg(5)	3.448(2)	3.468(2)	3.483(2)	3.492(2)	3.496(2)	3.514(2)	3.552(1)
	3	Cg(7)...Cg(8)	3.481(2)	3.502(2)	3.508(2)	3.511(2)	3.519(2)	3.535(2)	3.577(1)
	4	Cg(10)...Cg(11)	3.457(2)	3.478(2)	3.498(2)	3.501(2)	3.503(2)	3.517(2)	3.550(1)
	5	Cg(13)...Cg(14)	3.517(2)	3.537(2)	3.546(2)	3.553(2)	3.562(2)	3.575(2)	3.613(1)
		Average	3.472	3.493	3.508	3.513	3.519	3.535	3.571
$Z' = 1$		Cg(1')...Cg(2')	–	–	–	–	–	–	3.620(1)

\*Cg corresponds to the ring plane defined by atoms; for  $Z' = 5$ , Cg(1) = N1, C1, C2, N2, C8, C7; Cg(2) = C3, C4, C5, C6, C7, C8; Cg(4) = N3, C9, C10, N4, C16, C15; Cg(5) = C11, C12, C13, C14, C15, C16; Cg(7) = N5, C17, C18, N6, C24, C23; Cg(8) = C19, C20, C21, C22, C23, C24; Cg(10) = N7, C25, C26, N8, C32, C31; Cg(11) = C27, C28, C29, C30, C31, C32; Cg(13) = N9, C33, C34, N10, C40, C39; Cg(14) = C35, C36, C37, C38, C39, C40 and for the  $Z' = 1$ ; Cg(1') = N(1), C(3), C(4), N(2), C(10), C(9); Cg(2') = C(5), C(6), C(7), C(8), C(9), C(10).

**Figure 2.** Structural overlay of unit cells of the  $Z' = 1$  and the modulated  $Z' = 5$  form of quinoxaline viewed down the  $b$ -axis. Symmetry-independent molecules are shown in different colours ( $Z' = 1$ : black;  $Z' = 5$ : red, yellow, blue, green and magenta). The pseudo-translation in the  $Z' = 5$  structure is one-fifth the length of the long axis.

compared to the  $Z' = 1$  structure. Similar cases have been observed in which the most dense structure does not correspond to the thermodynamically most stable form<sup>11,12</sup>.

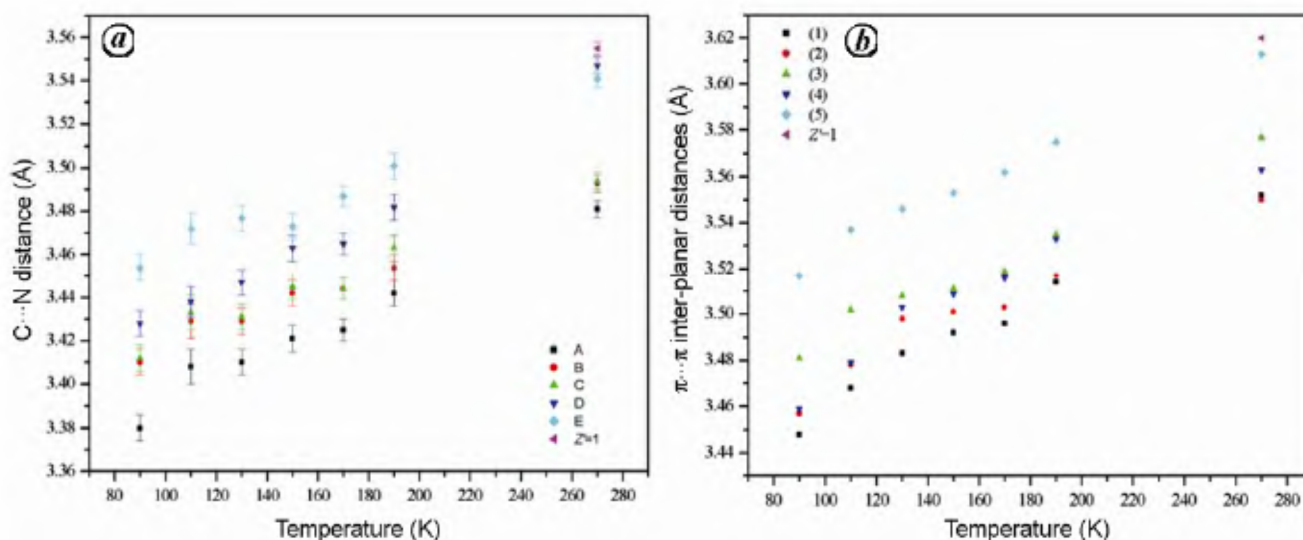
**Figure 3.** Molecular packing in quinoxaline  $Z' = 1$  crystal structure; **a**, C–H...N interactions between the molecules related by a  $2_1$  screw along  $b$ -axis and **b**,  $\pi \cdots \pi$  stacking interaction between the phenyl ring and the N-heterocyclic rings.

Modulated high  $Z'$  structures may be observed by kinetically trapping the molecular conformations or orientations by lowering the temperature. Recently, we observed a similar situation for the anti-HIV drug, efavirenz<sup>12</sup>. The molecule contains a conformationally flexible cyclopropylethynyl fragment. Form I of the drug ( $Z' = 3$ ) has the three symmetry-independent molecules with three distinct conformations about the triple bond. Cooling of form I

**Table 4.** Comparison of lattice energies for quinoxaline (Dreiding) to show relative contributions from the van der Waals and electrostatic terms\*

Structure	Temperature (K)	$E_{\text{vdW}}$ (kcal/mol)	$E_{\text{Elec}}$ (kcal/mol)	$E_{\text{Total}}$ (kcal/mol)
$Z' = 5$	90	-58.794	-4.401	-63.20
	110	-60.007	-4.379	-64.39
	130	-60.657	-4.378	-65.04
	150	-61.210	-4.345	-65.55
	170	-61.612	-4.335	-65.95
	190	-62.391	-4.296	-66.69
	270	-64.091	-4.149	-68.24
$Z' = 1$	270	-65.053	-3.967	-69.02

\*Valence energy terms were excluded from the total energy calculations for comparison. Calculations were performed with H-atom positions normalized to the neutron values. Atomic charges used in the calculation were obtained with the Gasteiger method.



**Figure 4.** Temperature dependence of the various (a) C–H...N interactions (A–E; see Table 2) and (b) interplanar distances for the  $\pi \cdots \pi$  stacking interactions (1–5; see Table 3) in the  $Z' = 5$  structure of quinoxaline (esd's are shown in the figure as error bars). The fact that the distances decrease in both cases with decreasing temperature shows that the interactions are of a stabilizing nature.

crystals leads to a further fixing of conformers with an increase in the  $Z'$  value to six, showing both better interactions and higher crystal density. In quinoxaline, trapping pertains to molecular orientation rather than conformation. The quinoxaline  $Z' = 5$  structure represents a case of modulation arising from kinetically trapped molecular orientations. These orientations are stabilized by shorter C–H...N interactions. However, no single crystal to single crystal phase transition to the  $Z' = 1$  structure was observed at elevated temperatures. From the above observations, we conclude that the crystals of  $Z' = 5$  are favoured by fast kinetics. Probably, a high activation barrier between the two structures does not allow a transformation of the high  $Z'$  structure to the  $Z' = 1$  structure prior to melting.

This work illustrates the theme of kinetic and thermodynamic polymorphs in crystallization. The kinetic polymorph is characterized by better intermolecular interactions

and the thermodynamic polymorph by a lower energy. Generally, the latter also has a higher density and packing coefficient, but this is not the case for the title compound in the present study. The crystal structures of the two forms of quinoxaline are closely related. In both forms, the molecules are held together by C–H...N hydrogen bonds. The  $Z' = 5$  form is a modulated structure with the value of the  $c$ -axis being five times that in the  $Z' = 1$  form. The variable temperature structure determinations show that the C–H...N interactions in both forms are true hydrogen bonds: they contract upon cooling. However, the hydrogen bond in the  $Z' = 1$  form is longer than any of the corresponding five symmetry-independent distances in the  $Z' = 5$  form. When the kinetic and thermodynamic forms of a crystal are distinct, polymorphism is a possibility. This is why polymorphs of quinoxaline can be isolated.

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Supplementary information: For details of the crystal structures, refer to the following CCDC deposition numbers in the Cambridge Crystallographic Data Centre: 792115–792121. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/products/csd/request/>

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## Long-term effects of early maternal separation and isolation stress on adulthood behaviour of female rats

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**The present study demonstrates the long-term effect of early maternal separation (EMS) and isolation stress on the adult emotionality behaviour of female rats. The maternal separation (MS) of rat pups constituted both separation and isolation from the littermates for three days from post-natal days 5–7 (stress hyporesponsive period, MS(SHRP)) and 16–18 (post-stress hyporesponsive period, MS(PSHRP); 6 h/day) respectively. SHRP is characterized by reduced capacity to secrete stress hormone under stressful situations, which is postulated to be essential for the normal development of hypothalamic–pituitary–adrenal axis. A control group consisted of rat pups never handled or separated from the mother. At post-natal day 61, the rats were exposed to a light/dark test, exploratory activity in a novel environment and passive avoidance test. Both control and MS(PSHRP) groups did not differ in the latency to enter into the dark compartment, number of transitions between light and dark compartments and total motor activities in the preferred dark chamber. However, MS(SHRP) rats exhibited increased activity in the dark chamber in the light/dark test. When exposed to a novel environment, MS(PSHRP) groups exhibited significant decrease in the freezing response when compared to both control and MS(SHRP) groups. Furthermore, following exposure to a passive avoidance test, both MS groups showed decreased latency to enter into the preferred chamber with reduced locomotor activity in the dark compartment, indicating stress-induced decreased attention as a consequence of EMS stress.**

**Keywords:** Anxiety, locomotor activity, maternal separation, stress, passive avoidance.

EARLY post-natal period is a critical phase for brain development, characterized by an enormous capacity for structural and functional reorganization of the neural circuitry in rodents. Any experiences and perturbations during this early period of life are thought to have lasting changes on brain functions and on behaviour during adulthood<sup>1,2</sup>. Aversive experiences during the developmental period can induce time-dependent influence on

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