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Neonatal cholinergic lesions and development of exploration upon administration of the GABAa receptor agonist muscimol in preweaning rats

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Abstract

Neonatal rats were administered 192 IgG-saporin (192 IgG-Sap), a selective cholinergic immunotoxin, on postnatal day (PND) 7. Behavioural responsiveness to muscimol, a GABAa receptor agonist, was then assessed using locomotor activity and object exploration tests on PND 18. In Experiment 1, 192 IgG-Sap-lesioned and control rats were injected with the GABAa agonist, muscimol, on PND 18 and tested in a standard open field test. Muscimol reduced rearing responses in both control and 192 IgG-Sap-lesioned animals whereas reduced wall-rearing responses occurred in control animals only. 192 IgG-Sap also reduced rearing and wall-rearing responses. In Experiment 2, muscimol effects were evaluated on PND 18 in a spatial open field test in which object exploration in addition to locomotion and rearing responses was assessed. Neonatal cholinergic lesion per se increased locomotion during object exploration while decreasing time spent exploring objects. Depressant effects of muscimol on object exploration were also evident. As a whole, these data provide evidence for (i) basal forebrain (BF) cholinergic control on locomotor activity and object exploration and (ii) GABAa-mediated regulation of selective behavioural patterns associated with locomotion and exploration in weaning rats. Neonatal cholinergic lesions, however, do not appear to alter reactivity to GABAergic agonists in juvenile rats.

Keywords: Exploratory behaviour; Muscimol; Developing rats; Spatial open field test; Wall-rearing; Rearing

1. Introduction

The cholinergic basal forebrain (BF) system is important in the development and maturation of the neural circuitry that is critical for learning, memory, exploration and response to novelty in adult rodents (Everitt and Robbins, 1997; Giovannini et al., 2001). The last decade has seen the emergence of more selective means of destroying BF cholinergic neurones, the immunotoxin 192 IgG-saporin (192 IgG-Sap). This toxin consists of a monoclonal antibody, 192 IgG, that is directed against rat p75 nerve growth factor receptor (NGFr), which is coupled with the ribosome-inactivating protein saporin. The 192 IgG-Sap exploits the fact that most cholinergic BF neurones express high levels of the NGFr relative to other cholinergic and noncholinergic neurones in nearby regions (Williams, 1971; Yan and Johnson, 1988). When injected intracerebroventricularly, 192 IgG-Sap selectively destroys cholinergic BF cells by inhibition of protein synthesis while sparing nearby non-cholinergic neurones both in adult and developing rats (Book et al., 1994; Leanza et al., 1996; Wiley, 1992; Wiley et al., 1991). 192 IgG-Sap, however, also targets cerebellar Purkinje cells in the adult rat brain (Heckers et al., 1994; Nilsson et al., 1992; Waite et al., 1994, 1995); by contrast, no Purkinje cells loss has been reported after neonatal treatments (Leanza et al., 1996), probably because of time courses of cerebellar 192 IgG expression (Eckenstein, 1988).

Whereas a large number of behavioural studies investigated the functional role of the cholinergic BF system in the adult rat brain (McGaughy et al., 2000; Wrenn and Wiley, 1998), considerably fewer studies focused on its role during development. Such ontogenetic studies have shown that 192 IgG-Sap treatment in the first postnatal week induced marked and long-lasting cholinergic depletion (Leanza et al., 1996; Pappas et al., 1996; Ricceri et al., 1997). By

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contrast, behavioural long-term effects following neonatal 192 IgG-Sap were not evident in the water maze and only mild-working memory deficits appeared in radial maze performance (Leanza et al., 1996; Pappas et al., 1996, 2000; Ricceri et al., 1999). However, selective deficits in spatial discrimination capabilities were evident when using a modified version of the open field test in which objects are placed in the experimental arena. Adult rats treated with 192 IgG-Sap on postnatal day (PND) 7 did not react to spatial rearrangements of familiar objects as compared to control rats, whereas responses to totally novel objects were comparable in neonatally lesioned and control rats (Ricceri et al., 1999). Altogether, data from neonatal 192 IgG-Sap administrations revealed that the cognitive deficits induced by cholinergic dysfunction are markedly age and task dependent (Ricceri et al., 1999). Interestingly, neonatal cholinergic lesions also affected early explorative behaviour: wall-rearing responses—a behavioural component of a general exploratory pattern in a novel environment—are significantly inhibited in preweaning rats after 192 IgG-Sap injections on either PND 7 or PND 1 and 3 (Ricceri et al., 1997, 2002a).

Neurochemical, pharmacological and behavioural data have shown that selective lesions of the cholinergic BF have also repercussions on other neurotransmitter functions. In the adult rat brain, cholinergic BF activity regulates glutamatergic (Fadel et al., 2001), serotoninergic (Lehmann et al., 2002) and GABAergic functions in cortical as well as hippocampal regions. Rossner et al. (1995) showed that 1 week after 192 IgG-Sap intracerebroventricular injection, NMDA receptor binding was reduced by about 15–20% and 40% in cortical and hippocampal regions, respectively, while binding to GABAa receptors increased by about 18–20% in cortical regions. The same binding profile has been observed in hippocampal regions. By contrast, the cholinergic lesion did not affect benzodiazepine receptor binding (Rossner et al., 1995).

It is also known that GABAergic and cholinergic neurones interspersed in the medial septum area strongly interact in regulating cognitive behavioural responses in adult rats. Combined lesions of GABAergic and cholinergic septal neurones induce memory impairments greater than those observed after selective lesions to either GABAergic or cholinergic population alone (Pang et al., 2001). Furthermore, responsiveness to a GABAa agonist (muscimol) is increased following medial septum saporin lesions in adult rats performing a T-maze alternation task (Pang and Nocera, 1999).

The role played by GABAergic system in regulating behavioural development as been primarily investigated in experiments using GABA agonists and antagonists. Some behavioural responses in rodent pups appear to be targeted by GABA agonists, including suckling responses in 3–4-day-old rats (Spear et al., 1986) and wall climbing and head rising and locomotion (age-specific motor responses) in neonatal mice (Laviola and Alleva, 1990; Ricceri et al., 2001; Tirelli, 1989). A larger body of evidence, however, has extensively documented an involvement of GABAergic system on modulation of pain reactivity both in developing and adult rodents (Balerio and Rubio, 2002; Laviola and Alleva, 1990; Ricceri et al., 2001; Sawinok, 1987).

In the present study, we intended to evaluate whether early disruption of the BF cholinergic neurones could result in altered GABAergic development in the same BF region. Our hypothesis was that some of the behavioural endpoints considered, namely, those associated with exploration patterns, could be differentially affected by GABAa stimulation in rats with neonatal BF lesions. Two different experiments were performed. In Experiment 1, neonatal rats were injected with 192 IgG-Sap on PND 7 and they underwent a standard open field test on PND 18 after injection of either muscimol 0.1 or 0.5 mg/kg ip. To evaluate the modulatory role of GABA on nociception, a 60-s hot plate test was also performed immediately after the open field test. In Experiment 2, neonatal rats underwent the same cholinergic lesion on PND 7 and they were then assessed in a modified version of the open field test (with objects) on PND 18 after injection of either muscimol 0.05 or 0.1 mg/kg ip. This task allows one to measure baseline locomotor activity, object exploration, habituation profiles and response to a mild change in the experimental environment (rearrangement of familiar objects in the arena). Importantly, previous data have extensively shown that performance in this task is sensitive to BF cholinergic dysfunction during development and at adulthood (Buhot et al., 1989; Poucet, 1989; Ricceri et al., 1999).

2. Experiment 1

2.1. Materials and methods

2.1.1. Animals and breeding procedures

Wistar rats were purchased from Charles River Italy (Calco, Italy) and kept in air-conditioned room at 21 ± 1 °C and $60 \pm 10\%$ relative humidity, with a white–red light cycle (white light on from 8:30 a.m. to 8:30 p.m.). Males and multiparous females were housed in couples in $42 \times 27 \times 14$ cm Plexiglas cages with metal tops and sawdust bedding. Pellet food (enriched standard diet purchased from Mucedola, Settimo Milanese, Italy) and tap water were continuously available. Two weeks after their arrival, rats were grouped into 16 breeding pairs. After 10 days, the females were individually housed and inspected daily at 9:30 a.m. for delivery (PND 1).

Litters were culled at birth to six males and two females; both sexes were left in the litter to avoid maternal behaviour biases, as well as long-term effects on the offspring, induced by the presence of pups of one sex only (Laviola and Terranova, 1998). Six male pups in each group (for a total of 36 animals) were used in the experiment.

2.1.2. Treatments

The male pups in each litter were randomly assigned to either the vehicle (phosphate-buffered saline, PBS, 0.1 M, $n=3$ in each litter) or 192 IgG-Sap (Chemicon, Temecula CA, USA, $n=3$ in each litter) treatment condition. On PND 7, between 9 and 12 a.m., pups were removed from their mothers and anesthetised by hypothermia. Pups to be lesioned were secured in a stereotaxic apparatus (Stoelting, USA) with a Plexiglas mold holder for neonate rats. The 192 IgG-Sap (Chemicon International, Temecula, CA, USA; Batch #20021123) was injected over 1 min using a 30-gauge needle that was left in place for an additional 1 min. A pair of injections (192 IgG-Sap 0.42 μg, dissolved in 1 μl of PBS on each side) were therefore made at the following coordinates: AP = 0.0; ML = ± 2.0; DV = − 3.5 relative to bregma. Control pups received a similar injection of 1 μl of PBS on each side. Previous study performed on 7-day-old rats showed that this procedure was effective in administering 192 IgG-Sap into the third ventricle (Ricceri et al., 1999). After surgery, animals were sutured with Histoacryl tissue glue (Braun, D-34209, Melsugen, Germany), transferred to a heating pad for 20 min to regain normal body temperature and subsequently returned to their home cages.

On PND 18, three male pups in each group (PBS or 192 IgG-Sap) from each litter were randomly assigned to one of three treatment conditions saline solution (0.9% NaCl) or two doses of muscimol chloride (0.1 and 0.5 mg/kg; Sigma Aldrich, St. Louis, MO, USA) injected intraperitoneally in a volume of 1.0 ml/g body weight. The two muscimol doses were selected on the basis of previous experiment in developing rodents (Laviola and Alleva, 1990; Spear et al., 1986). All experimental procedures used were in compliance with the EC guidelines (EC Council Directive 86/609, 1987) and the Italian legislation (Decreto L.vo 116/92) on animal experimentation.

2.1.3. Standard open field test

The apparatus was an open field arena (35 × 35 × 35 cm) made of black Plexiglas, with the floor subdivided into squares of 7 × 7 cm each. A pup from each treatment (PBS and 192 IgG-Sap groups) was injected intraperitoneally 20 min before testing. At the beginning of the test, each pup was individually placed in one corner of the arena and its behaviour was video recorded for 20 min. The following responses were then measured: crossing (crossing the square limits with both forepaws); rearing (standing on hind legs); wall rearing (standing on hind legs and placing forelimbs on the wall of the arena); grooming (wiping, licking, combing or scratching of any part of the body) and inactivity (no visible movements). The Observer (Wageningen, NL; Noldus, 1991), a software system for collection and analysis of observational data, was used for scoring the duration and frequency of each response, except for crossing, for which only the frequency was measured.

2.1.4. Hot plate test

Immediately after the open field test, nociceptive responses of animals were evaluated in a hotplate apparatus (Model D837 Socrel, Basile, Comerio, Italy). Briefly, each animal was individually placed in the centre of the apparatus delimited by a 19-cm diameter glass cylinder and maintained at 50.0 ± 0.1 °C (cutoff time is 60 s, for general methodology in the use of the hotplate test, see Cirulli et al., 2000). Nociceptive heat sensitivity was assessed by scoring latency time and frequency of the following behavioural items: forepaw licking (f-licking) and hindpaw licking (h-licking) and wall rearing. The session was video recorded under red light, and data collection from the videotapes was performed using the Observer software.

2.1.5. Statistical analysis

A mixed-model analysis of variance (ANOVA) with repeated measures was used to analyse open field and hot plate data (frequency and duration), always considering the litter as a random variable. Litters are therefore considered statistical units, and data from control and treated littermates are "repeated trials" of the litter unit (Zorrilla, 1997). We performed post hoc comparisons by using Tukey's honestly significant difference (HSD) test, which can be used in the absence of significant ANOVA results (Wilcox, 1987). Nonparametric ANOVA (Friedman test) was used to analyse latency data from the hot plate test.

2.2. Results

The high muscimol dose (0.5 mg/kg) resulted cataleptic for both control and cholinergic-lesioned pups and was therefore excluded from the analysis.

2.2.1. Open field testing

Analysis of frequency of crossings did not show a significant main effect of either 192 IgG-Sap or muscimol; data, however, suggest that muscimol tended to decrease locomotor activity in PBS-treated animals (mean number of crossings ± S.E.M.: PBS–SAL = 77.7 ± 13.1; PBS–MUSC = 52.5 ± 11.5) and to enhance locomotion in 192 IgG-Sap animals (192 IgG-Sap–SAL = 56.1 ± 16.4; 192 IgG-Sap–MUSC = 91.9 ± 18.7), with the interaction 192 IgG-Sap × Muscimol just missing statistical significance [$F(1,5)=4.58$, $P=.08$].

Wall-rearing frequencies are shown in Fig. 1 (upper panel). A significant interaction 192 IgG-Sap × Muscimol was found [$F(1,5)=7.8$, $P=.03$]; post hoc comparison performed on this interaction revealed that muscimol decreased wall rearing only within the PBS group ($P<.05$) likely because 192 IgG-Sap decreased baseline expression of this behaviour (PBS–SAL vs. 192 IgG-Sap, $P<.05$). As for wall-rearing duration (Fig. 1, upper panel), the main effect of 192 IgG-Sap treatment just missed statistical significance [$F(1,5)=5.4$, $P=.06$], whereas the interaction 192 IgG-Sap × Muscimol was not significant [$F(1,5)=1.0$].

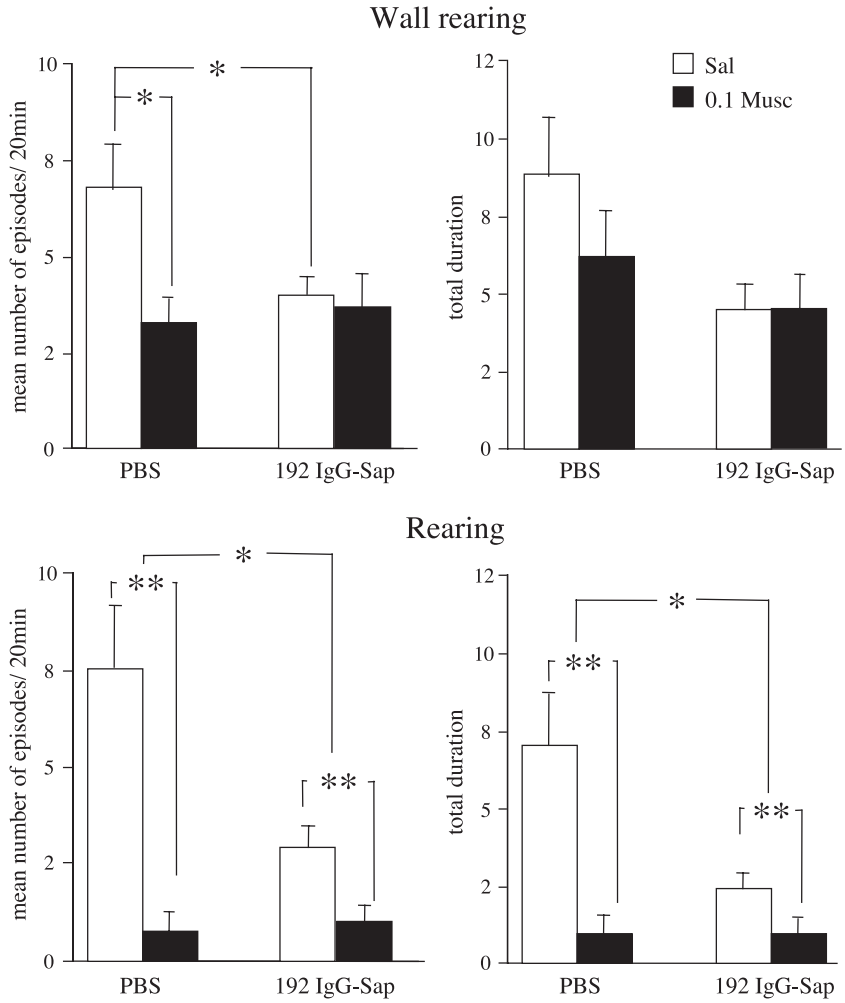

Fig. 1. Experiment 1: Frequency and duration of wall-rearing and rearing responses by 18-day rats during a 20-min open field test. Rats underwent either lesion of the BF neurones with the immunotoxin 192 IgG-Sap or PBS control treatment on PND 7 and they were injected intraperitoneally with either saline or 0.1 mg/kg muscimol 20 min prior to the beginning of the test. Data are means ± S.E.M. * indicates $P < .05$, ** indicates $P < .01$ after post hoc comparisons.

As for rearing responses (Fig. 1, lower panel), both frequency and duration were decreased by 192 IgG-Sap treatment [frequency $F(1,5) = 7.0$, $P = .04$; duration $F(1, 5) = 8.3$, $P = .03$]. In addition, muscimol significantly decreased rearing responses in both PBS and 192 IgG-Sap rats [frequency $F(1,5) = 12.2$, $P = .01$; duration $F(1,5) = 11.8$, $P = .01$]. Also, interaction 192 IgG-Sap × Muscimol was significant [frequency $F(1,5) = 7.4$, $P = .04$; duration $F(1,5) = 9.6$, $P = .02$].

No significant differences among treatments were evident in grooming and immobility.

2.2.2. Hot plate test

Hot plate data are shown in Table 1. Analysis of latency data (Friedman test followed by multiple comparisons) did not reveal any significant effect of 192 IgG-Sap or muscimol. An analgesic effect of the GABAergic agonist was, however, evident when considering the duration of h-licking, which decreased in muscimol-treated pups [$F(1, 5) = 6.9$, $P = .04$], whereas the muscimol effect just missed statistical significance on licking frequency [$F(1,5) = 5.6$, $P = .06$]. No effect of the 192 IgG-Sap treatment or 192 IgG-Sap × Muscimol was evident on frequency and duration of hindlimb and forelimb licking and wall rearing.

2.3. Discussion

As a whole, these results indicated that in control animals, the GABAa stimulation primarily affected motor/exploratory responses (reduction of wall rearing and rearing responses) and nociception, as shown by decrease in h-licking responses in muscimol-treated pups. This analgesic effect of muscimol is fully in line with previous observations in developing rodents (Laviola et al., 1992; Ricceri et al., 2001) and did not result altered by neonatal BF cholinergic lesion.

As for the behavioural effects of the neonatal cholinergic lesion per se, a significant reduction of both wall- and open-rearing responses was evident, in agreement with previous data (Ricceri et al., 1997, 2002a). The reduction of wall

Table 1
Selected behavioural responses during the hot plate test (50 °C)

	Control		192 IgG-Sap	
	Saline	0.1 Muscimol	Saline	0.1 Muscimol
Hindlimb licking (*f*)	2.3 ± 1.0	0.3 ± 0.3	1.3 ± 0.8	0.3 ± 0.2
Hindlimb licking (*d*)	5.1 ± 2.2	0.4 ± 0.4	3.1 ± 1.6	0.5 ± 0.3 *
Hindlimb licking (*l*)	37.4 ± 16.6	59.2 ± 3.8	48.5 ± 9.5	54.4 ± 10.2
Forelimb licking (*f*)	1.3 ± 0.6	0.5 ± 0.2	0.8 ± 0.3	1.2 ± 0.5
Forelimb licking (*d*)	3.2 ± 1.4	2.0 ± 1.1	1.6 ± 0.6	5.8 ± 4.6
Forelimb licking (*l*)	43.9 ± 12.3	60.0 ± 1.9	55.2 ± 8.7	60.0 ± 6.6
Wall rearing (*f*)	3.2 ± 1.2	2.0 ± 0.4	4.6 ± 0.8	1.6 ± 0.4
Wall rearing (*d*)	4.2 ± 1.6	4.0 ± 1.2	5.0 ± 1.0	4.6 ± 1.6
Wall rearing (*l*)	20.0 ± 23.9	47.0 ± 15.2	26.0 ± 7.1	45.6 ± 15.8

Data are mean frequencies (*f*) and durations (*d*) ± S.E.M.
Data are median ± interquartile for latency; $n = 6$ in each group.
* Denotes a significant main effect of muscimol $P < .05$.

rearing induced by 192 IgG-Sap was likely responsible for the apparent lack of responsiveness to GABAergic agonist after the lesion. In other words, while muscimol was able to decrease the wall-rearing response baseline of PBS-treated animals, it could not reduce the already lowered baselines in cholinergic-lesioned animals.

3. Experiment 2

Experiment 2 was aimed at evaluating, in greater detail, the role of GABAergic regulations on motor/explorative responses in control and cholinergic-lesioned animals. We therefore used a spatial open field test since results from Experiment 1 suggested that environmental exploration components of the behavioural repertoire more than simple locomotor activity levels could evidence direct muscimol effects in preweaning rats and the possible interactions of this treatment with neonatal cholinergic lesion.

3.1. Materials and methods

3.1.1. Animals, breeding procedures and treatment

In this experiment, we used animals who were born in excess in another study carried out in our laboratory in which the effects of the cholinergic lesion were assessed at the adult stage (Ricceri et al., 2002b). This strategy allowed us to reduce the total number of animals used in experimentation, but rats came from different litters and we could not take into account the litter random variable. Breeding procedures were as in Experiment 1, and surgery was performed on PND 7, as previously described. On the basis of the results of Experiment 1, we used lower muscimol doses, namely, 0.05 and 0.1 mg/kg, injected 20 min prior to the beginning of the spatial open field test. A total of 47 animals were used (PBS–SAL, $n = 9$; PBS–LowM, $n = 8$; PBS–HighM, $n = 9$; SAP–SAL, $n = 7$; SAP–LowM, $n = 8$; SAP–HighM, $n = 6$).

3.1.2. Spatial open field test

3.1.2.1. Apparatus. The apparatus was a black circular open field arena, 120 cm in diameter with a 30-cm high wall made of plastic material. The arena was placed into a soundproof cubicle and surrounded by a visually uniform environment except for a striped pattern (20 × 10 cm) attached to the wall of the arena. The apparatus was illuminated only by a red light (80 W) located on the ceiling to favour exploration of the novel environment (Williams, 1971). A video camera above the field was connected to a video recorder.

Three different objects were simultaneously present in the open field: (A) a plastic grey cylinder with a metal grid wall (height, 15 cm; diameter, 13 cm); (B) a plastic black cylinder with a metal grid wall (height, 12 cm; diameter, 8 cm) and (C) a transparent Plexiglas cube with holes irregularly distributed on the sides (height, 10 cm).

3.1.2.2. Behavioural procedure. Open field tests were performed between 9:30 a.m. and 2:30 p.m. Rats were individually submitted to four successive 5-min sessions, separated by 3-min delays, during which the subjects were returned to their home cage. During Session 1 (S1), each rat was placed into the empty open field to become familiar with the apparatus and to record a baseline level of locomotor activity. During Sessions 2 and 3 (S2–S3), the objects were placed in the arena, and in Session 4 (S4), the spatial test session, the configuration was changed by moving two objects: objects C and A were exchanged.

3.1.2.3. Data collection. Data collection was performed using a video recorder and the Observer software. During the first session, the frequencies of the following responses were measured: rearing, wall rearing and crossing (crossing the annulus in which the floor of the arena was subdivided on the monitor). From S2 to S4, object exploration was measured as time spent by the animal in contact with an object. A contact was defined as the subject's snout actually touching an object. Habituation to the objects was assessed by averaging the duration of contacts with the objects during S2 and S3 in each group. In S4, the spatial arrangement of the objects was modified. Such object displacement is expected to increase object exploration during S4 in adult, but not weaning rodents (Ricceri et al., 2000). Because of different levels of object exploration during S3 among the experimental groups, an object exploration index was used to compare object exploration during S4; it consists of the increase of time spent exploring objects in S4 divided by S3 exploration time [(S4 − S3)/S3]. Data collection was performed by an investigator unaware of the treatment.

3.1.3. Statistical analysis

A mixed-model ANOVA with repeated measures was used to analyse open field data. We performed post hoc comparisons by using Tukey's HSD test, which can be used in the absence of significant ANOVA results (Wilcox, 1987).

3.2. Results

3.2.1. S1 (no objects)

Neither effect of the neonatal treatment with 192 IgG-Sap [$F(1,41) = 1.74$, *ns*] nor of the two muscimol doses [$F(1,41) = 1.74$, *ns*] was evident in locomotor activity scores.

Frequency of wall rearing and rearing was also analysed during S1. The reduction induced by neonatal cholinergic lesion on number of wall-rearing episodes just missed statistical significance [$F(1,41) = 3.25$, $P = .07$]; also, the main effect of muscimol just missed statistical significance [$F(2,41) = 2.65$, $P = .08$]. Post hoc comparisons performed on the interaction Cholinergic Lesion × GABAergic Agonist [$F(2,41) = 2.15$, $P = .12$] revealed that the low muscimol dose induced an increase of wall-rearing responses in control but not in cholinergic-lesioned animals ($P < .05$; see Fig. 2), thus suggesting a decreased responsiveness to the 0.05 muscimol dose after neonatal cholinergic lesion. As for rearing frequencies, ANOVA did not show a significant main effect of either 192 IgG-Sap [$F(1,41) = 0.17$] or GABAergic agonist [$F(2,41) = 1.45$], but data still suggest that muscimol tended to decrease rearing in both control (mean number of episodes ± S.E.M.: PBS–SAL = 1.55 ± 4.3; PBS–LowM = 0.50 ± 1.06; PBS–HighM = 0.22 ± 0.44) and cholinergic-lesioned rats (SAP–SAL = 1.14 ± 1.46; SAP–LowM = 0.37 ± 1.06; SAP–HighM = absence of response).

3.2.2. S2–S4 (with objects)

In S2 and S3, when three objects were placed in the arena, ANOVA revealed a significant increase of locomotion in cholinergic-lesioned animals [$F(1,40) = 6.06$, $P = .01$]. Post hoc comparisons performed on the interaction Cholin-

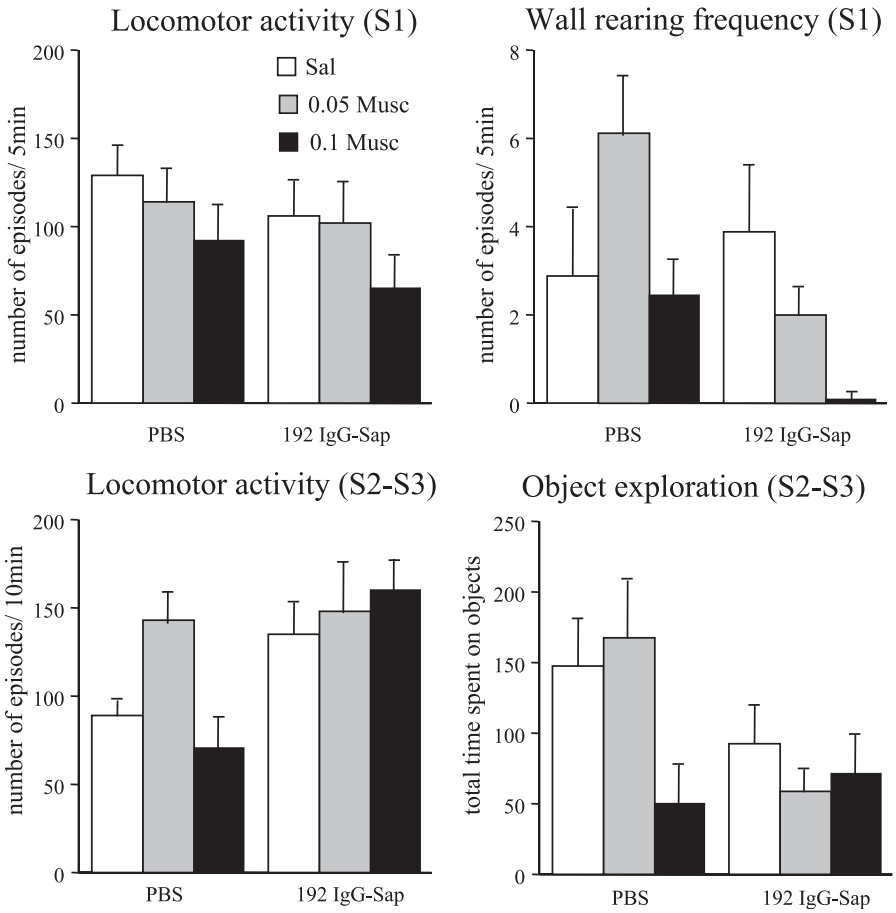

Fig. 2. Experiment 2: Different behavioural items displayed in a spatial open field test (four sessions of 5 min each) by 18-day rats. Rats underwent either lesion of the BF neurones with the immunotoxin 192 IgG-Sap or PBS control treatment on PND 7 and they were injected intraperitoneally with saline, 0.05 mg/kg (0.05 Musc) or 0.1 mg/kg (0.1 Musc) 20 min prior to the beginning of the test. Upper panel: frequency of crossings (a general locomotor activity measure) and wall rearing during the first session of the test (5-min). Lower panel: frequency of crossings and time spent in contact with the objects during S2 and S3. Data are means ± S.E.M. The effect of the cholinergic lesion resulted significant for crossings ($P = .01$) whereas just missed to be statistically significant for object contact.

ergic Lesion × GABAergic Agonist revealed that increased locomotion was primarily evident in 192 IgG-Sap 0.1 mg/kg muscimol group.

As for object exploration, 192 IgG-Sap treatment tended to decrease the time spent in contact with objects, such effect missing statistical significance [$F(1,38) = 3.27$, P=.07]. Post hoc comparisons performed on the interaction Cholinergic Lesion × GABAergic Agonist [$F(2,38) = 2.02$, P=.14] revealed a significant difference in exploration between control and 192 IgG-Sap animals injected with the low muscimol dose ($P < .05$).

Reactivity to the spatial rearrangement occurring in S4 was evaluated by means of an index of object exploration [(time spent on objects on S4 − time spent on objects on S3)/time spent on objects on S3]. ANOVA did not show any significant effect of the cholinergic lesion [$F(1,38) = 1.44$], GABAergic agonist [$F(2,38) = 0.61$] or their interaction [$F(2,38) = 1.30$] (PBS−SAL = − 0.01 ± 0.24; PBS−LowM = − 0.29 ± 0.18; PBS−HighM = 0.64 ± 1.02; SAP−SAL = 2.9 ± 2.62; SAP−LowM = 1.26 ± 0.99; SAP−HighM = − 0.24 ± 0.30). As expected in 3-week-old animals, rats failed to respond with increased exploration of displaced objects to the spatial rearrangement of the test environment. When object exploration was evaluated in S4 only, displaced objects (A and C) appear to be explored as much as the nondisplaced one (object B); interestingly, ANOVA revealed a significant main effect of GABAergic agonist [$F(2,41) = 4.85$, P=.01] due to a depressant effect of object exploration of the HighM dose occurring in both control and 192 IgG-Sap animals (PBS−SAL = 55.2 ± 14.6; PBS−LowM = 58.6 ± 17.9; PBS−HighM = 19.4 ± 7.8; SAP−SAL = 55.3 ± 21.1; SAP−LowM = 58.6 ± 11.3; SAP−HighM = 16.1 ± 6.7).

3.3. Discussion

Results from Experiment 2 indicated that the use of a behavioural test focused on object exploration revealed a hyperactivating effect of the neonatal cholinergic lesion only evident after introduction of objects in the arena and not in the initial phase of the open field test.

In agreement with locomotor activity data, the hyperactive-lesioned animals also tended to spend less time exploring the objects when compared to PBS controls. This lesion effect prevented the possibility of detecting any further GABAa-mediated inhibition of exploration, apart from a reduction of object exploration in S4, induced by the high muscimol dose.

As for wall-rearing data, they apparently did not confirm results of Experiment 1 since no effect of the 0.1 mg/kg muscimol dose was observed, and a paradoxical increase of wall rearing was induced by the lower muscimol dose (0.05 mg/kg) in control but not in cholinergic-lesioned rats. Direct comparisons of wall- and open-rearing responses from the two experiments could, however, be misleading since the duration of the test significantly differed (20 min in Experiment 1 vs. a 5-min session in Experiment 2). Indeed, in Experiment 2, wall-rearing responses were recorded only in S1 because placement of objects in the arena significantly biased the occurrence of the wall-rearing response (animals tend to make "object rearing" instead of wall rearing).

This effect of the low muscimol dose on wall rearing is, however, in full agreement with previous findings: an activating effect of low muscimol doses (and no or inhibiting effect of higher doses) has already been reported in developing rodents on different locomotor responses in rodent pups (Spear et al., 1986; Laviola and Alleva, 1990; Ricceri et al., 2001).

4. General discussion

4.1. Behavioural effects of the GABAergic agonist

Results from Experiment 1 confirm the reported developmental role of GABAergic pathways in rat nociception. Besides this well-known analgesic response, muscimol also inhibits selected locomotor/explorative responses. Indeed, results from Experiment 1 show that systemic administration of GABAa agonist decreased rearing and wall-rearing responses that are relevant behavioural components of exploratory patterns in a novel environment. Results from Experiment 2 show that, in a different test more focused on object exploration, the same dose of GABA agonist decreased object exploration on S4.

As a whole, our results suggest a GABAa-mediated regulation of exploratory patterns in rodents at weaning. So far, in adult rodents, only locomotion was analysed as a behavioural endpoint sensitive to GABA dysfunction. We are not aware of detailed data on effects of muscimol on environmental exploration. Present data suggest that (i) exploration could be a behavioural endpoint sensitive to GABA alteration in the adult rat brain too (and this should be taken into account when performing cognitive tests with explorative/motor components) and (ii) a detailed analysis of the several behavioural responses displayed in the open field test, rather than computerised locomotor activity scores, can reveal the effective magnitude of the GABAergic regulation in the development of exploratory behaviour.

Our data, pointing to exploratory endpoints as a behavioural marker of GABAergic function during development, are not fully comparable with those previously reported because of substantial differences in age ranges and associated behavioural repertoire considered. The available data, however, reported a general suppression of different spontaneous behavioural responses after GABAa agonist administration both in neonatal rats (Spear et al., 1986) and in 2–3-week old mice (Laviola and Alleva, 1990; Nagy et al., 1979; Ricceri et al., 2001). GABA agonist treatment also attenuate d-amphetamine-induced increases of locomotion, wall climbing and head rising in 11-day-old mice (Tirelli, 1989).

4.2. Behavioural effects of the neonatal BF lesion

The removal of cholinergic input to neocortex and hippocampus at the end of the first postnatal week also appears to decrease locomotor/explorative responses in the standard open field test (Experiment 1) and during the spatial open field with objects (Experiment 2). The neonatal lesion appears to target more consistently wall- than open-rearing responses, thus confirming both previous results from 192 IgG-Sap experiments (Ricceri et al., 1997, 2002a) and those data differentiating between the neural regulation subserving wall- and open-rearing responses during development (Linville and Spear, 1988; Scalzo and Burge, 1992) and in adulthood (Clifford et al., 1998; Russell et al., 1987). Indeed, data from adult rats strongly indicate a selective dopamine (D1A mediated) regulation of open rearing and grooming but not of wall rearing (Clifford et al., 1998).

Unfortunately, the direction of the behavioural effects induced by the neonatal BF lesion does not favour the identification of possible interactions with the subsequent GABAa agonist treatment. Indeed, it appeared difficult to evidence the inhibitory action of GABAa agonist on a baseline value already decreased by the BF lesions, and most of the differential effects of muscimol here reported can be easily interpreted as ''floor effects''. Not surprisingly, a comparison with the few data available on GABAergic function after BF lesion in adulthood leads us to conclude that the effects of early 192 IgG-Sap administration are substantially different from those induced by the same lesion in an adult brain where increased GABAa binding in the cortex and hippocampus (Rossner et al., 1995) and behavioural responsiveness to intraseptal muscimol injections have been reported (Pang and Nocera, 1999). Since latter data, however, have been collected following intraseptal muscimol injections and we cannot exclude that the differences between neonatal versus adult interactions between GABA and Ach systems could be due to different route of administration of muscimol used.

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