Using Athymic Nude (FOXN1^{n}) Mice as a Model to Study the Maternal Care and Levels of Ultrasonic Vocalization

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Abstract

The correlation between the numbers of ultrasounds produced by pups and the different measures of retrieving behavior of their mother was investigated using athymic nude mice (*Foxn1^{n}*) and their heterozygous thymus-bearing littermates (*Foxn1^{n}/Foxn1^{+}*) as a models. Significantly higher latencies of retrieve *Foxn1^{n}* as compared to *Foxn1^{n}/ Foxn1^{+}* pups on 1-pnd (postnatal day) were reported, while no differences on 3-pnd were detected. Many ultrasound vocalization parameters were investigated but the more important results were: 1) While no differences in alone group (every model alone in the cages with their biological mother), significant higher latency of start calling and average call duration of *Foxn1^{n}/Foxn1^{+}* as compared to *Foxn1^{n}* in together group (both models together in the same cages) were detected. On the other hand, significant lower latency of start calling of *Foxn1^{n}* pups on 2-pnd in together observed as compared to that in alone group, 2) Higher call numbers and total calling time were detected in *Foxn1^{n}/Foxn1^{+}* as compared to *Foxn1^{n}* in together and alone groups on 2-pnd, and 3) While no differences in together group, statistically significant higher medium frequency modulation and bandwidth were reported in *Foxn1^{n}/Foxn1^{+}* as compared to *Foxn1^{n}* in alone group on 2-pnd.
These results suggested that the maternal retrieving and USV emission of pups may be affected by genotype. The most differences were detected on 1-, 2-pnd but not on more than 3-pnd. These findings could indicate an early communication deficit in nude mice.

Keyword: Athymic (nude) mice, vocalization, ultrasound produced, maternal care, behavior.

Introduction

Early life experience has a long-lasting influence on the development of neural systems implicated in emotion and cognition (Gross and Hen, 2004; Zhang *et al*., 2006). Behaviorally, ultrasonic vocalizations have been proposed as a useful measure of anxiety in infant and adult rodents (Wohr and Schwarting, 2008). These vocalizations occur during stressful situations in rats and mice, such as hunger (Jans and Leon, 1983), cold (Bell *et al*., 1972), handling (Bell *et al*., 1971), and unfamiliar orders (Oswalt and Meier, 1975).

Ultrasound production is considered to have a communication function essential for the pups` survival (Noirot, 1972). This author and others have stressed the implications of ultrasound production in retrieving or maternal behavior, as follows. Pups are unable to thermo-regulate from birth to 6 days of age, and consequently, maternal retrieving of the pups to the nest and subsequent lactating position is necessary to ensure a constantly high body temperature. Visual and acoustic stimulation provides the distal cues that initiate retrieving and maternal behavior. However, Smotherman *et al*. (1974) demonstrated that the presence of auditory cues elicited shorter retrieving latencies only when the female was aroused by olfactory cues. Experiments with inbred strains of mice also challenged the conclusions about auditory cues maintaining maternal behavior. A positive interstrain correlation was obtained between the numbers of ultrasounds produced by pups and the different measures of retrieving behavior of their mother (Roubertoux et al., 1996).

A spontaneous mutation resulting in athymic mice were first described in 1966 (Flanagan, 1966; Segre et al., 1995). Mutation in the nude locus produce the remarkable pleiotropic phenotype of hairlessness and athymia (Flanagan, 1966; Pantelouris, 1968). The nomenclature to design nude mice has changed several times since its discovery. The most actual one was introduced in 2000 by Kaestener and colleagues when the gene responsible for the mutation was identified as a member of the Fox gene family, and the

nomenclature was updated to *Foxn1*^{n} (Kaestner *et al*., 2000).

Because of the anthemia, nude mice lack T lymphocyte functions (Loor and Kindred, 1973; Raff, 1973). Later, there is good evidence that the athymic mice, because of the athymia, have many differences in brain proteins contains such as Noradrenalin (NA), Brain derived nerve growth factor (BDNF), and Nerve growth factor (NGF) (Jouda *et al*., 2012). Some of these proteins like BDNF and NGF play an important role in the development and maintenance of the nervous system (Barde, 1989), and it is expected that these differences can change the mice behaviors. For example, it is known that the BDNF participates in several hippocampus function, including spatial learning and memory (Mizuno *et al*., 2000), and adult neurogenesis (Scharfman *et al*., 2005). In other hand, we observed, that the mothers (*Foxn1*^{n}/ *Foxn1*$^{+}$) care at the *Foxn1*^{n}/ *Foxn1*$^{+}$ babies more than the *Foxn1*^{n} babies and because of that most of *Foxn1*^{n} babies die when they still with another babies (*Foxn1*^{n}/ *Foxn1*$^{+}$) in one cage. Congenitally athymic nude mice offer an excellent model to study the ultrasound production which considered to have a communication function essential for the pups` survival and the maternal behavior of the mothers.

Materials and Methods

Animals: In the animals house of physiology and pathophysiology institute of medical college of Philipps University in Marburg in Germany the male athymic (Balb/C *Foxn1*^{n}) and female (Balb/C *Foxn1*^{n}/ *Foxn1*$^{+}$) were bred under constant temperature, humidity in light (12hs light-dark cycles) controlled room. They were fed *ad libitum*. The pups, males and females; *Foxn1*^{n} and *Foxn1*^{n}/ *Foxn1*$^{+}$, were housed together with their biological mother. Six mice cages containing mothers with their babies were used in maternal care test on 1-, and 3-pnd. These mice cages were used with other ten mice cages: five mice cages contain mothers with their babies (*Foxn1*^{n}), and five cages mice contained mothers with their babies (*Foxn1*^{n}/ *Foxn1*$^{+}$) in ultrasonic vocalization test on 2-, 4-, 6-, 8-pnd.

Maternal care: On 1-pnd and 3-pnd the mother and the pups were removed from the housing cage without destruction of the nest. The markings of the pups were refreshed. After approximately 5 min of separation, pups were scattered over the floor of the housing cage and the mother was reintroduced. Latencies to retrieve every pup were measured. Observations ended when all pups were retrieved, or when 5 min. had elapsed, that is, a ceiling score of 5 min. was used when a mother did not retrieve all pups.

Ultrasonic vocalizations: On 2-; 4-; 6-; and 8-pnd, under room temperature (23-24˚C), between 08:00–14:00 h, during the light phase of the 12:12 h light/dark cycle, pups were removed individually from the nest at random and gently placed into an isolation container (10x8x7 cm; open surface) made of glass, containing clean bedding material. The isolation container was surrounded by a sound attenuating box (17x17x19 cm) made of cork (thickness of the walls: 6 cm). USV emission was monitored by an Ultra-Sound Gate Condenser Microphone GM 16 placed in the roof of the sound attenuating box, 10 cm above the floor. The microphone was connected via an Ultra-Sound Gate 116 USB audio device to a personal computer, where acoustic data were recorded with sampling pups of 250.00 Hz in 16 bit format by Avisoft RECORDER. The microphone used for recording was sensitive to frequencies of 15-180 kHz with a flat frequency response (±6 dB) between 25-140 kHz. After 5 min isolation period, body weights were determined and then Individual pups were not returned to the home cage until were labeled with ink marker hands.

For acoustical analysis, recordings were transferred to SAS Lab Pro and a fast Fourier transform was conducted (512 FFT length, 100% frame, Hamming window and 75% time window overlap). Correspondingly, the spectrograms were produced at 488 Hz of frequency resolution and 0.512 ms of time resolution. Call detection was provided by an automatic threshold-based algorithm (amplitude threshold: - 40 dB) and a hold-time mechanism (hold time: 10 ms). Since no USV was detected below 30 kHz, a high-pass filter of 30 kHz was used to reduce background

noise outside the relevant frequency band to 0 dB. The accuracy of call detection by the software was verified manually by an experienced user. When necessary, missed calls were marked by hand to be included in the automatic parameter analysis. Total number of USV was calculated for the entire session and in 60s time bins, to visualize the time course of the USV response. Additional parameters, based on previous studies of isolation-induced calling (Kurz *et a*l., 2010; Wohr *et al*., 2008; Wohr and Schwarting, 2008), included peak frequency and peak amplitude, i.e. loudness, which were derived from the average spectrum of the entire call, were determined automatically Fig. (1). Peak amplitude was defined as the point with the highest energy within the spectrum. Peak frequency was defined as the frequency at the location of the peak amplitude within the spectrum. In addition, the extent of frequency modulation, i.e. the difference between the lowest and the highest peak frequency within each call, was measured automatically. Temporal parameters included latency to start calling, calling numbers, total calling time, call duration, peak frequency, peak amplitude, band width, and medium frequency modulation.

Statistical analysis: Results are expressed as mean ± SE. Data were analyzed by one-way analysis of variance (ANOVA) followed by Fisher`s test for multiple comparisons, using StatView version 5.0. Differences were considered significant at $p<0.05$.

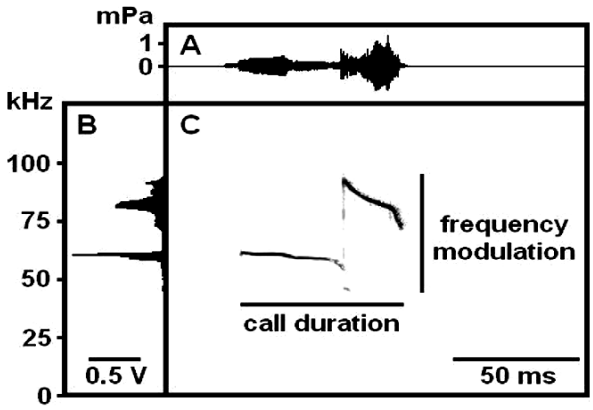

Fig.(1) Analysis of ultrasonic vocalizations.

Energy within the spectrum is shown by time (A) and frequency (B) and time x frequency (C, reflected as ‘‘darkness’’**)**. Peak amplitude, i.e. loudness, was defined as the point with the highest energy within the spectrum (‘‘darkest’’ points over time in C). Peak frequency was defined as the frequency at the location of the peak amplitude within the spectrum (‘‘darkest’’ points over time in C). Peak frequency and peak amplitude were derived from the average spectrum of the entire call, meaning that values obtained per time point were averaged over time. The extent of frequency modulation was defined as the difference between the lowest and the highest peak frequency within each call, i.e. derived from the non-averaged call (C). Temporal parameters were latency to start calling, total calling time, call duration (C).
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Results

Since it has been reported that athymic mice have an abnormal growth rate as compared to normal mice, the body weight was recorded. The results of these determinations are shown in Table (1). The body weight was significantly lower in athymic mice than in their heterozygous thymus-bearing littermates at nearly all ages studied and in both together and alone groups.

While no difference was detected on 3-pnd Fig.(2B), statistically significant ($P<0,05$) higher latencies of retrieve was observed in *Foxn1*^{n} pups as compared to *Foxn1*^{n}*/ Foxn1*$^{+}$ littermates on 1-pnd Fig.(2A). These results were matched our observation that the mothers care at *Foxn1*^{n}*/ Foxn1*$^{+}$ pups was more than *Foxn1*^{n} littermates, but the pup that survive until 3-day-old will be live.

Table (1)
body weight of athymic Foxn1^n, Foxn1^n/ Foxn1$^+$.

		Body weight (g)	
Group type	**PND (days)**	**Foxn1^n**	**Foxn1^n/ Foxn1$^+$**
Together group	2	1,59 ± 0,04	1,74 ± 0,03*
	4	2,27 ± 0,63	2,78 ± 0,72
	6	3,07 ± 0,73	3,99 ± 0,91*
	8	3,83 ± 0,77	5,17 ± 0,73*
Alone group	2	1,64 ± 0,05	1,87 ± 0,02*
	4	2,29 ± 0,41	2,68 ± 0,32*
	6	3,32 ± 0,78	3,66 ± 0,74
	8	4,22 ± 0,98	4,56 ± 0,94

Values are explained as mean ± SE. Statistically significant differences: * *Foxn1^n/ Foxn1$^+$* vs *Foxn1^n* within the same group.

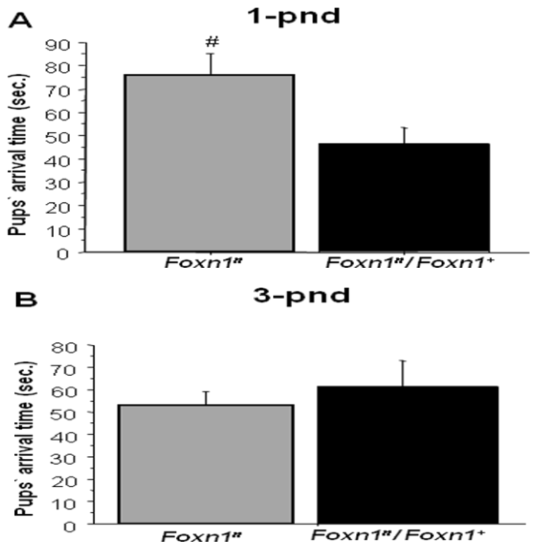

Fig.(2) The latencies of retrieve athymic pups on 1-pnd and 3-pnd.

Latencies to retrieve (pups´ arrival time) were determined to athymic nude-*Foxn1^n* pups (n=15) and their heterozygous thymus-bearing littermates- *Foxn1^n/Foxn1^n* (n=17) at different ages. (A) on 1-pnd, (B) on 3-pnd. # Statistically significant difference from *Foxn1^n/Foxn1$^+$*at (P<0,05).

The differences in latency of start calling of *Foxn1^n/Foxn1$^+$* and *Foxn1^n* in together group on 2-, 6-, and 8-pnd were statistically significant (P<0,05). The same tendency was observed in 4-pnd Fig. (3A) but no significant differences were observed between them in alone group Fig.(3B).

Essentially, the same differences were observed in the average call duration. While no difference in alone group Fig.(3D), significantly (P<0,05) higher call duration was recorded in *Foxn1^n/Foxn1$^+$* as compared to *Foxn1^n* in together group on 2-pnd, 6-pnd, and the same tendency was observed on 4-, and 8-pnd Fig.(3C).

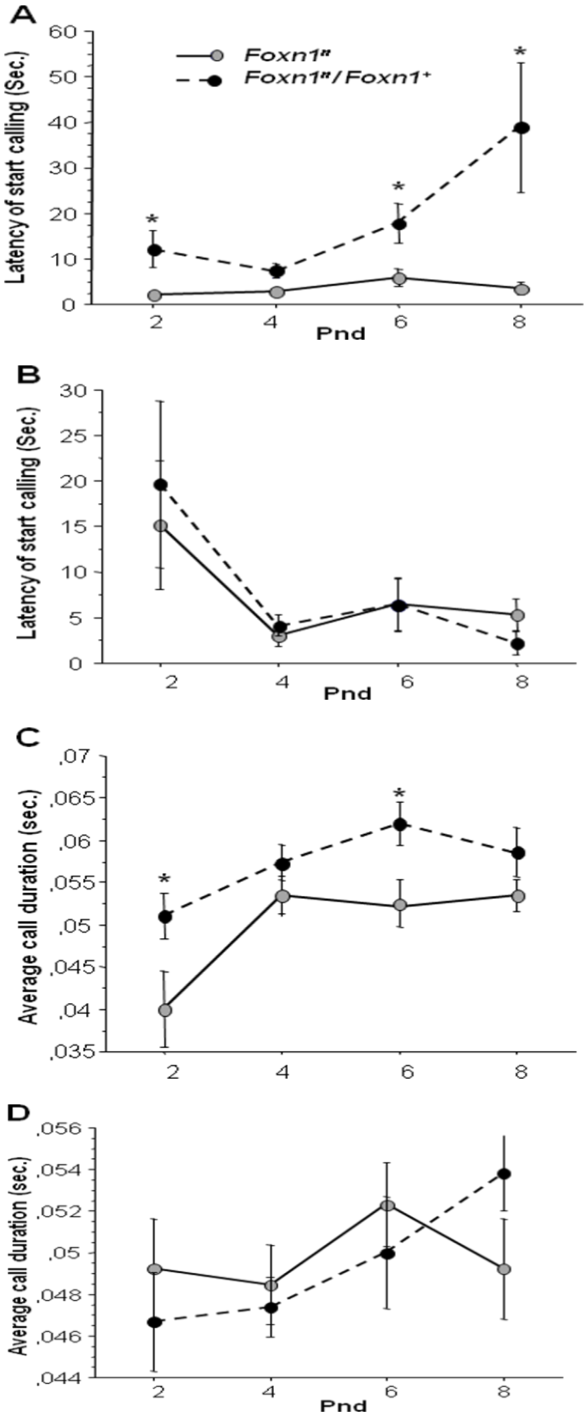

Fig.(3) The latency of start calling and average call duration of Foxn1^n/Foxn1$^+$ and Foxn1^n in alone and together groups.

Latency of start calling and average call duration were determined in alone group to *Foxn1*^{n} (n=13) and *Foxn1*^{n}/*Foxn1*^{n} (n=9), and together group to *Foxn1*^{n} (n=9) and *Foxn1*^{n}/*Foxn1*^{n} (n=9) at different ages. (A&B) Latency of start calling, (C&D) average call duration, (A&C) alone group, (B&D) together group.

*Statistically significant difference from *Foxn1*^{n} (P<0,05).

In Fig.(4), the latency of start calling and average call duration of *Foxn1*^{n} pups on 2-pnd in alon group were significantly (P<0,05) lower than them in together group.

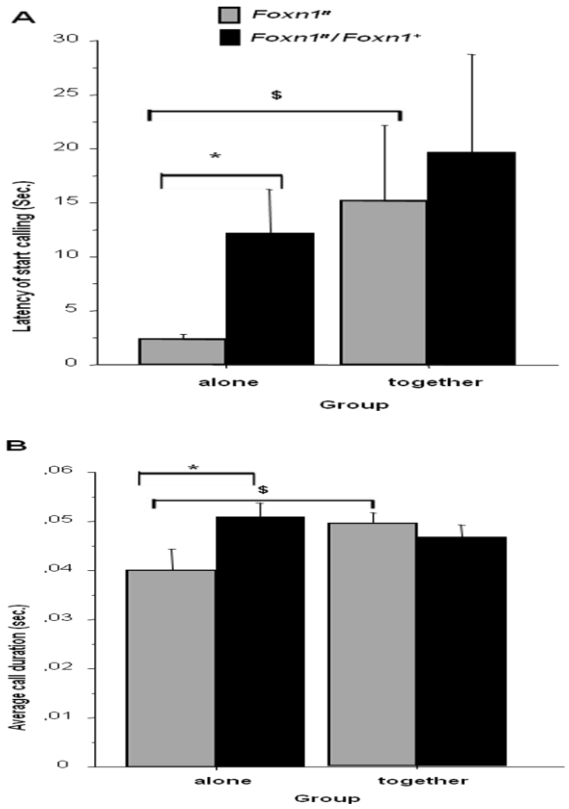

Fig.(4) The latency of start calling and average call duration of Foxn1^{n} and Foxn1^{n}/Foxn1^{+} pups on 2-pnd in alone and together groups.

(A) Latency of start calling, (B) average call duration. *Statistically significant difference from *Foxn1*^{n}, $ Statistically significant difference in *Foxn1*^{n} from together at P < 0,05.

These results suggested that the *Foxn1*^{n} and *Foxn1*^{n}/ *Foxn1*$^{+}$ pups start to call at the same time when they together but when they alone *Foxn1*^{n} start first, and *Foxn1*^{n} in together group start to call very early than them in alone group. The *Foxn1*^{n} pups have less average call duration than the *Foxn1*^{n}/ *Foxn1*$^{+}$ pups in alone group but this average increase in together group.

Statistically significant (P<0,05) higher call numbers were detected in *Foxn1*^{n}/*Foxn1*$^{+}$ pups as compared to *Foxn1*^{n} in both together and alone groups only on 2-pnd Fig. (5A & B). Comparable results were obtained in Total calling time, the differences were also statistically significant (P<0,05) on 2-pnd in together and alone groups Fig. (5C & D).

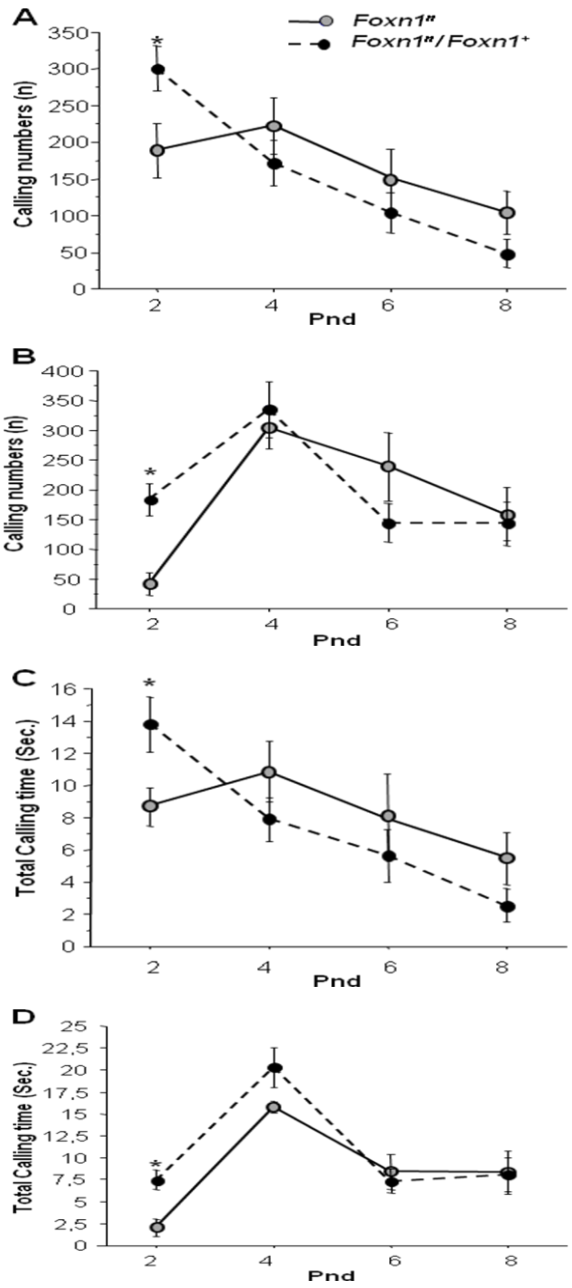

Fig.(5) The call numbers and total calling time of Foxn1^{n}/Foxn1^{+} and Foxn1^{n} in both together and alone groups.

Call numbers and total calling time were determined in alone group to *Foxn1*^{n} (n=13) and *Foxn1*^{n}/*Foxn1*^{n} (n=9), and together group to *Foxn1*^{n} (n=9) and *Foxn1*^{n}/*Foxn1*^{n} (n=9) at different ages. (A&B) Call numbers, (C&D) total calling time, (A&C) together group, (B&D) alone group. *Statistically significant difference from *Foxn1*^{n} at P<0,05.

In Fig.(6), the call numbers of *Foxn1*^{n} and of *Foxn1*^{n}/*Foxn1*$^{+}$ pups on 2-pnd in together group were significantly (P<0,05) higher than them in alone group Fig.(6A). Essentially, the same differences were observed in total calling time. Significantly (P<0, 05) higher total calling time were noticed in *Foxn1*^{n} and *Foxn1*^{n}/*Foxn1*$^{+}$ pups on 2-pnd in together group as compared to them in alone group Fig.(6B).

These results suggested that the *Foxn1*^{n}/ *Foxn1*$^{+}$ pups on 2-pnd call their mother more than the *Foxn1*^{n} pups and the *Foxn1*^{n} and *Foxn1*^{n}/ *Foxn1*$^{+}$ pups call their mother more when they together than when they alone.

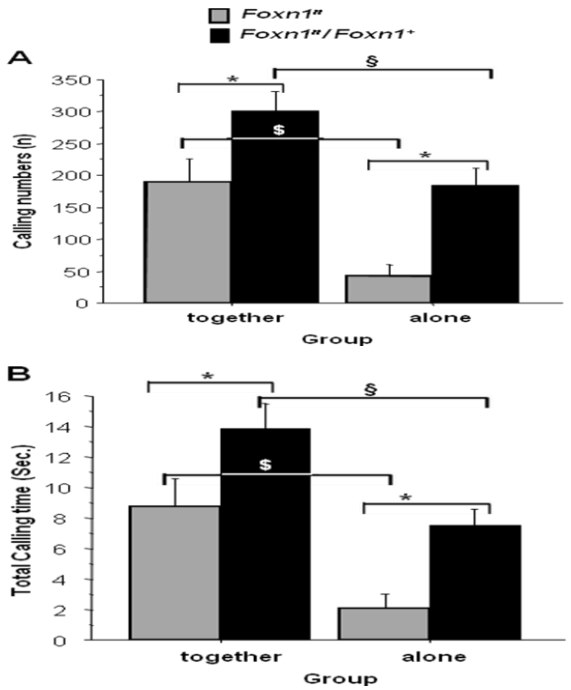

Fig.(6) The call numbers and total calling time of Foxn1^{n}/Foxn1^{+} and Foxn1^{n} in together group and alone groups.

(A & B) call numbers, (B) total calling time. *Statistically significant difference from *Foxn1*^{n}, $ Statistically significant difference in *Foxn1*^{n} from together, § Statistically significant difference in *Foxn1*^{n}/*Foxn1*$^{+}$ from together group at P<0,05.

No significant differences were detected in peak frequency and peak amplitude of *Foxn1*^{n}/*Foxn1*$^{+}$ and *Foxn1*^{n} in both together group and alone groups Fig.(7). In dicating that the peak frequency and peak amplitude were not affected by genotype.

While no significant differences were detected in the medium frequency modulation in together group Fig.(8A), statistically significant (P<0,05) higher medium frequency modulation was detected in *Foxn1*^{n}/*Foxn1*$^{+}$ as compared to *Foxn1*^{n} in alone group only on 2-pnd Fig.(8B). Comparable results were obtained in bandwidth. Statistically significant (P<0,05) differences between bandwidth of *Foxn1*^{n}/*Foxn1*$^{+}$ and of *Foxn1*^{n} were detected only in alone group on 2-pnd Fig.(8D), but no significant difference in bandwidth in together group was detected Fig.(8C).

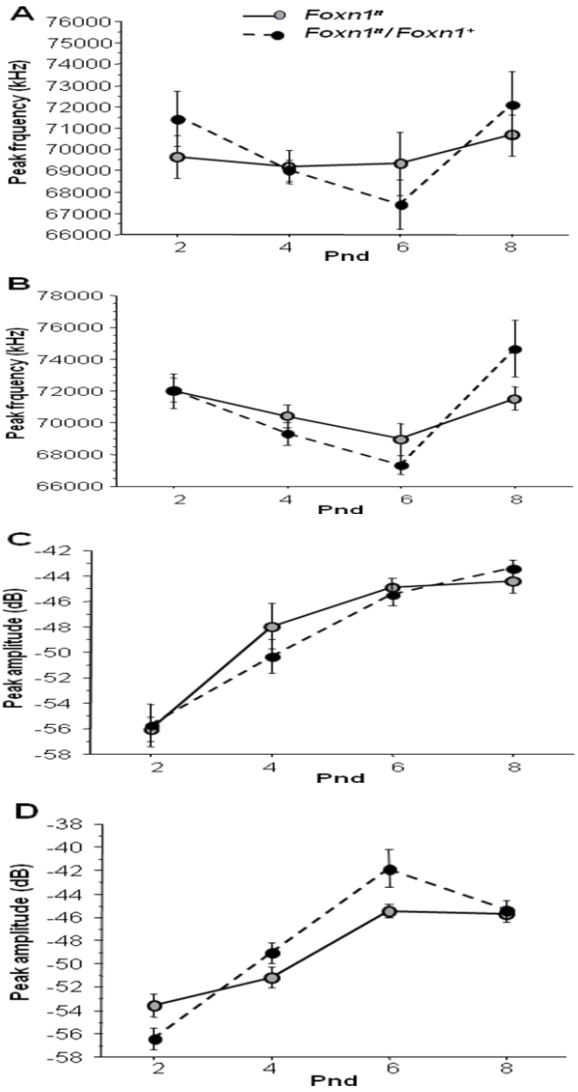

Fig.(7) The peak frequency and peak amplitude of Foxn1^{n}/Foxn1^{+} and Foxn1^{n} in both together and alone groups.

Peak frequency and amplitude were determined in alone group to *Foxn1*^{n} (n=13) and *Foxn1*^{n}/*Foxn1*^{n} (n=9), and together group to *Foxn1*^{n} (n=9) and *Foxn1*^{n}/*Foxn1*^{n} (n=9) at different ages. (A&B) Peak frequency, (C&D) peak frequency, (A&C) together group, (B&D) alone group.

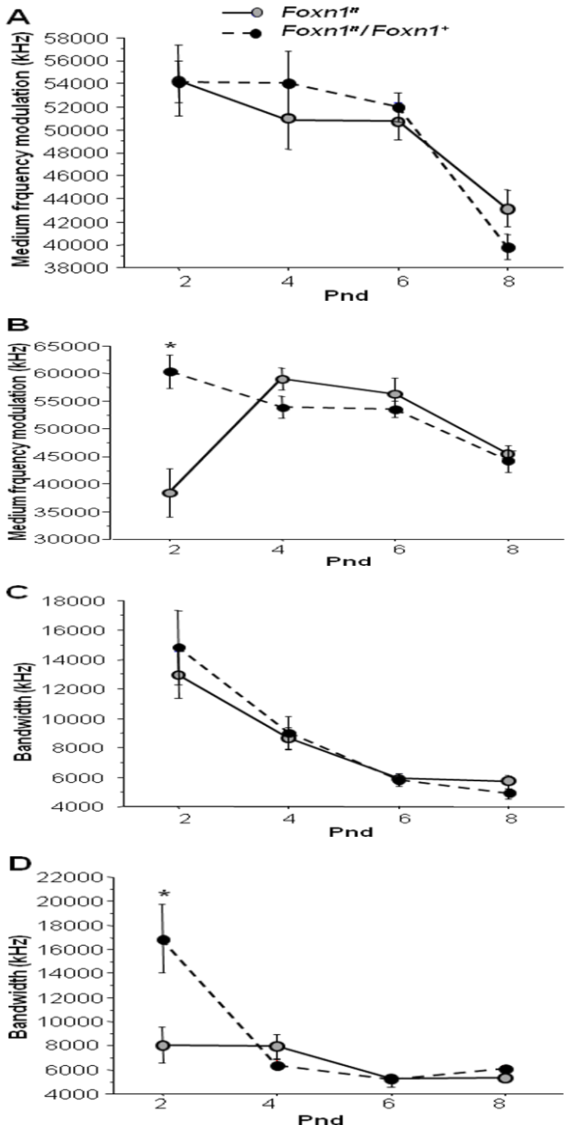

Fig.(8) The medium frequency modulation and Bandwidth of Foxn1^{n}/Foxn1^{+} and Foxn1^{n} in alone and together groups.

Medium frequency modulation and Bandwidth were determined in alone group to *Foxn1*^{n} (n=13) and *Foxn1*^{n}/*Foxn1*^{n} (n=9), and together group to *Foxn1*^{n} (n=9) and *Foxn1*^{n}/*Foxn1*^{n} (n=9) at different ages. (A&B) Medium frequency modulation, (C&D) bandwidth, (A&C) together group, (B&D) alone group. *Statistically significant difference from *Foxn1*^{n} at (P<0,05).

In Fig.(9), the medium frequency modulation and bandwidth of *Foxn1*^{n} on 2-pnd in alone group were significantly (P<0,05) lower than them in together group Fig.(9). These results suggested that the *Foxn1*^{n}/*Foxn1*$^{+}$ pups call their mother more strongly than the *Foxn1*^{n} pups on 2-pnd and the *Foxn1*^{n} pups call their mother more strongly when they together with their littermates *Foxn1*^{n}/*Foxn1*$^{+}$.

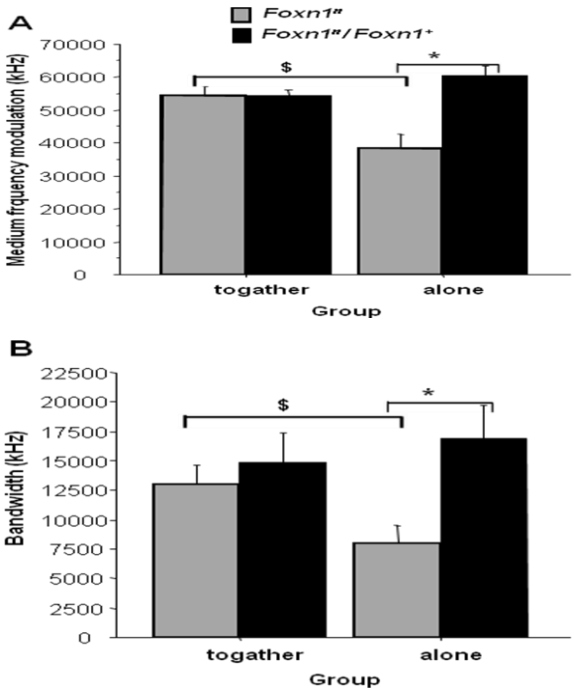

Fig.(9) The medium frequency modulation and Bandwidth of Foxn1^{n} and Foxn1^{n}/Foxn1^{+} in together and alone groups.

(A) Medium frequency modulation, (B) bandwidth. *Statistically significant difference from *Foxn1*^{n}, $ Statistically significant difference in *Foxn1*^{n} from together at (P<0,05).

Discussion

The interaction between the maternal care of the mother and the ultrasonic vocalization parameters and the genotype of the pups was studied. The vocalizations occur during stressful situations in rats and mice, such as hunger (Jans and Leon, 1983), cold (Bell *et al*., 1972), handling (Bell *et al*., 1971), and unfamiliar orders (Oswalt and Meier, 1975).

Communication between a mother and her young is an important constituent of maternal behavior and the ultrasonic vocalization was considered to have a communication function

essential for the pups` survival (Noirot, 1972). A positive inter strain correlation was obtained between the numbers of ultrasounds produced by pups and the different measures of retrieving behavior of their mother (Roubertoux *et al*., 1996).

The implications of ultrasound production in retrieving or maternal behavior have stressed, as follows. Pups are unable to thermo-regulate from birth to 6 days of age, and consequently, maternal retrieving of the pups to the nest and subsequent lactating position is necessary to ensure a constantly high body temperature (Noirot, 1972). Visual and acoustic stimulation provides the distal cues that initiate retrieving and maternal behavior. However, Smotherman *et al*.(1974) demonstrated that the presence of auditory cues elicited shorter retrieving latencies only when the female was aroused by olfactory cues. Experiments with inbred strains of mice also challenged the conclusions about auditory cues maintaining maternal behavior (Roubertoux *et al.* 1996).

There is compelling evidence that isolation-induced USV serve a communicative function. Pups calls elicit maternal search and retrieval behavior. A reduced level of calling or an unusual calling pattern has been reported in several mouse models, but not in *Foxn1*^{n} mice models (Ehret, 1987; Chadman *et al*., 2008; Young *et al*., 2010), which could be indicative of a communication impairment. Importantly, it was shown that less maternal care giving was directed to mouse pups that vocalized only rarely (Bell, 2010). Hung *et al*. (2008) reported that the reduced level of call production in mutated mouse pups was insufficient to elicit maternal care.

In the present study, the changes in USV emission over duration were highly dependent on genotype. While there was an increase in call number, total calling time, duration, frequency modulation, and bandwidth of calls in *Foxn1*^{n}*/ Foxn1*$^{+}$ pups, this increase was weaker or absent in *Foxn1*^{n} pups.

It appears possible that call parameters such as call duration, peak frequency, peak amplitude, and frequency modulation, affect the communicative value of USV. Playback studies have shown that lactating mice can distinguish between different call types, and that they prefer certain call types over others if given the choice (Ehret, 1992; Wohr *et al*., 2008). Smith (1976) showed that mothers prefer a call with an 80 ms duration over a call with a 15 ms duration. Ehret (1992) found that mothers respond to calls with durations higher than 30 ms, but not to shorter ones. With respect to peak frequency, mothers showed a stronger response towards a 65-45 kHz signal than to a 75-55 kHz signal (Smith, 1976). This is probably because the mouse auditory thresholds increase rapidly above 60 kHz (Ehret, 1974). Call amplitude seems also to be important to attract the mother. By means of a pup discrimination task where two vocalizing pups were presented, it was shown that mothers spent more time near pups emitting loud calls (Wohr *et al*., 2008). Finally, Bruzynski *et al* (1999) postulated that the level of frequency modulation could be important for the efficacy of maternal search and retrieval behavior. It may be easier for the mother to detect and localize a highly frequency modulated call than a steady sound at a constant frequency. Calls emitted by nude mouse pups were shorter, weakly, and less frequency modulated than ones emitted by normal thymus-bearing mouse pups, although they start first to call their mother. This means that all altered parameters of calls emitted by nude mouse pups may decrease their signal value and hence elicit less maternal care giving responses.

The decrease in the USV productions of *Foxn1*^{n} pups in alone group was completely normalized when the *Foxn1*^{n} pups were live together with their *Foxn1*^{n}*/ Foxn1*$^{+}$ littermates in one cage and also later in ontogeny (more than 3-pnd). Therefore, all call parameters, with the exception of peak frequency and amplitude, i.e. loudness, were either directly affected by genotype, or their temporal pattern was affected by genotype, this is particularly remarkable as the other two factor studied, body weight and chemical components of brain, had only minor effects on USV production.

Since a genotype difference was detected for body weight which matched with other researches (Besedovsky *et al*., 1987; Jouda *et al.*, 2012; Pantelouris, 1973), it is possible that the lower USV level in *Foxn1*^{n} pups is due to

their lower body weight. In light of the small genotype difference in body weight of only 0,25 grams, however, body weight appears unlikely to be the cause of the reduced USV level in *Foxn1*^{n} pups. Lack of correlation between body weight and USV emission further argues against an interpretation that genotype differences in call emission were due to differences in body weight.

Since a difference in the chemical component of brain, such as, BDNF, was detected in previous work (Jouda *et al*., 2012), which increase the memory and learning ability of the mouse (Mizuno *et al*., 2000), and adult neurogenesis (Scharfman *et al*., 2005), it is possible that the normalization of the call parameters alterations in the together group and on more than 3-pnd is caused by this increase in BDNF level in brain. This means these nude pups learning with the time how to call their mother and this learning was more clearly when they together with their normal littermates.

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الخلاصة

تم في هذا البحث دراسه العلاقه بين نتائج المسح فوق الصوتي لأصوات صغار الفئران حديثة الولاده وأختلاف قياسات سلوك ألأم مع تلك الصغار وعلاقة ذلك بالتغيرات الجينيه الموجوده لدى الصغار من خلال استخدام الفئران الفاقده للغده الزعتريه (التوته) او ماتدعى بالفئران العاريه او (*Foxn1*^{n}) حسب احدث تسميه أضافه الى اشقائها المالكين لجين الغده الزعتريه (*Foxn1*^{n}/*Foxn1*$^{+}$) كموديل للبحث. اظهرت النتائج حدوث ارتفاع معنوي لقياسات متوسط زمن أعادة ألأم للصغار (*Foxn1*^{n}) الى اعشاشها مقارنة بـ (*Foxn1*^{n}/*Foxn1*$^{+}$) في اليوم الاول مابعد الولاده 1-pnd, بينما لم يلاحظ اي اختلاف في اليوم الثالث بعد الولاده. تم التوصل الى الكثير من نتائج المسح فوق الصوتي لأصوات الصغار لكن اكثرها اهميه كانت كما يلي: 1) لم يكن هنالك اي اختلاف بين نوعي الصغار في المجموعه التي يكون فيها كل نوع جيني من الصغار بمعزل عن الاخر مع امه البايولوجيه, الا انه هنالك ارتفاع معنوي بمتوسط زمن بداية النداءات ومتوسط مدة هذه النداءات للصغار من نوع *Foxn1*^{n}/*Foxn1*$^{+}$ مقارنة بالنوع *Foxn1*^{n} في المجموعة التي يكون فيها كلا النوعين من الصغار معا في قفص واحد مع امهم البايولوجيه. 2) وجود ارتفاع معنوي في عدد مرات النداءات والمجموع الكلي للزمن الذي تستغرقه هذه النداءات للنوع *Foxn1*^{n}/*Foxn1*$^{+}$ مقارنة بـ *Foxn1*^{n} في كلا المجموعتين وفي اليوم الثاني مابعد الولاده.

3) عدم وجود اي اختلاف في المجموعة التي يكون فيها كلا النوعين الجينيين من الصغار معا في قفص واحد مع امهم البايلوجيه مع ارتفاع معنوي في متوسط التردد المتحور وعرض النطاق الترددي لنداءات النوع $Foxn1^{n}/Foxn1^{+}$ مقارنة بـ $Foxn1^{n}$ في المجموعه التي يكون فيها كل نوع جيني من الصغار بمعزل عن الاخر مع امهم البايولوجيه وفي اليوم الثاني بعد الولاده.

هذه النتائج تدل على ان رعاية الام لصغارها و القياسات فوق الصوتيه لنداءات الصغار حديثي الولاده تتأثر بشكل واضح بالنمط الجيني للصغار. أن اغلب التغيرات التي تم الحصول عليها لوحظت في اليوم ألأول والثاني بعد الولاده وليس اكثر من ذلك العمر مما يشير الى عجز الصغار من نوع $Foxn1^{n}$ (وهوالنوع الحاوي على الطفره الوراثيه) عن التواصل المبكر مع الاخرين ومع الام بشكل خاص.