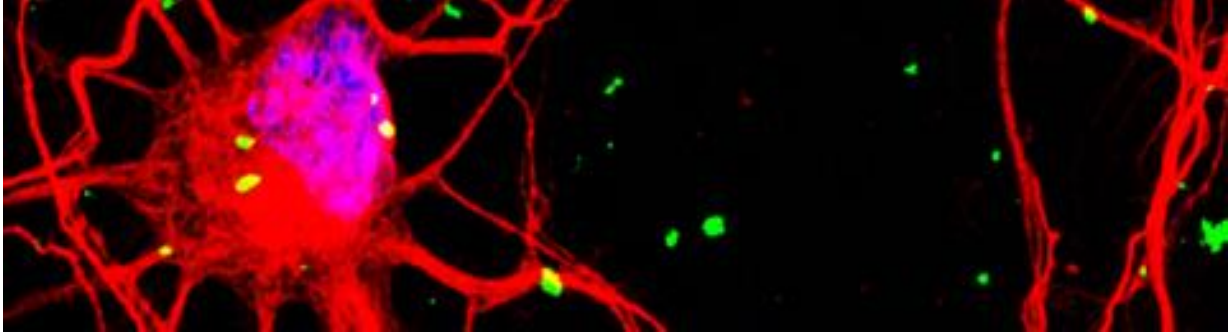

Corso di Dottorato in Neuroscienze
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Ciclo XXXI

Electrophysiological and behavioral characterization of parent-of-origin effects in mice

Autore: Ilaria Cosentini
Supervisor: Valter Tucci

ISTITUTO ITALIANO DI TECNOLOGIA

UNIVERSITÀ DEGLI STUDI DI GENOVA

Contents

Introduction

Sleep

Sleep is a complex behavior that occurs in all animals studied so far. Sleep can be defined as a reversible behavioral state in which awareness is reduced, as well as muscle activity and arousal threshold are higher (Rasch and Born, 2013). In humans as well as rodents, waking and sleep stages can be defined by electroencephalogram (EEG) combined with electromyography (EMG). Sleep is characterized by three different stages: wakefulness, rapid eye movement sleep (REM) and non-rapid eye movement sleep (NREM), which alternate cyclically. Each phase is characterized by specific patterns of brain activity and muscle tone. Sleep is a conservative phenomenon across species, as well as its circadian and neurochemical regulations; however, some aspects are different between sleep in humans and in rodents. The organization of sleep/wake cycle is in the opposite phase in rodents respect to humans. In fact, rodents are nocturnal animals; they are active during the dark phase and asleep during the light phase. Moreover, while in humans sleep is monophasic, meaning that, usually is present in only one session during a 24 hours period, in rodents is polyphasic and fragmented (Paterson et al., 2011; Toth and Bhargava, 2013).

As mentioned above, three different stages can be distinguished based on their specific features.

Wakefulness: during wake cortical neurons fire irregularly, EEG signal presents low-amplitude and high-frequency oscillations, with consequent activation of muscle tone (Brown et al., 2012) (Figure 1). From the behavioural point of view, during wake eyes are open and interaction with external environment is present.

REM sleep: also known as paradoxical sleep (PS), constitutes about 20% of total sleep time. In human sleep, REM sleep extends and intensifies during towards the end of the sleep period (Rasch and Born, 2013). EEG signal during REM sleep is characterized by low-amplitude and high-frequency brain oscillations (Figure 1), similar to what is observed during wakefulness. However, unlike waking, during REM sleep there is almost total muscle inactivity combined with phasic events, such as rapid eye movements and twitches of the limbs. For this reason, REM sleep can be briefly defined as a highly activated brain in a paralyzed body (Datta and O'Malley., 2013). During REM sleep, EEG is mainly characterized by theta oscillations (5-8 Hz) and slow alpha activity mostly originated in the hippocampus. However, theta oscillations have been found also in cortical structures even if these are not able to generate theta activity alone (Buzsaki, 2002). Moreover, REM sleep is associated with suspended thermoregulation, irregular breathing and heartbeats (Kalia, 2006) and dreaming (Scarpelli et al., 2015; Solms, 2000). REM sleep may also play a role in declarative memory (Kyriacou et al., 2010) and memory consolidation (Blissitt, 2001; Frank and Benington, 2006).

NREM sleep: also known as slow-wave sleep (SWS), NREM constitute about 80% of total sleep time. In humans it represents the predominant phase during the early part and decreases in intensity and duration during the sleep period (Rasch and Born, 2013). NREM sleep is characterized by regular occurrence of high-amplitude, slow wave oscillations in delta range (0.5-4.5 Hz) (Steriade, 2001), both locally and globally present (Vyazovskiy and Harris, 2013) (Figure 1). SWA is usually used as marker of NREM intensity and sleep pressure (Tononi and Cirelli, 2006; Vyazovskiy and Harris, 2013). For instance, the greater the waking period, the greater the spectral power in the slow wave range in the following sleep, which will decrease during the sleep episode (Achermann and Borbely, 2003). Moreover, it has been proposed that SWA is mainly involved in the restorative processes of sleep (Tononi and Cirelli, 2006; Vyazovskiy et al., 2000; Vyazovskiy and Harris, 2013).

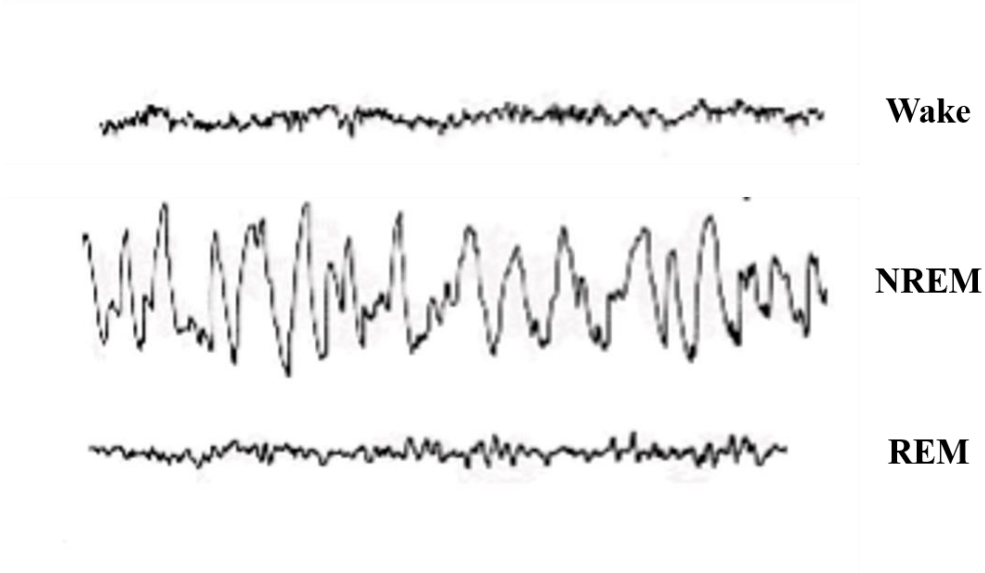


Figure 1. EEG pattern of wakefulness, NREM stages and REM sleep. (Adapted from Šušmáková K. 2004)

Regulatory mechanisms of sleep

Borbely (Borbely, 1982) proposed a model for sleep regulation that includes two main processes:

Circadian process (C), a self-sustained mechanism, controlled by an internal oscillator, located in the suprachiasmatic nucleus of the hypothalamus (SCN), above the optic chiasm, which represents the master biological clock, in mammals. SCN regulates the daily rhythms of the body and brain and it is not dependent on prior sleep-wake history. The oscillatory pattern of circadian activity has a period of approximately 24 hours, and this drive from the SCN also coordinates the activity of peripheral clocks of other organs to control all biological rhythms within the body (Richardson, 2005). Moreover, circadian

process actively modulates sleep propensity across hours of the day and both REM and NREM sleep are under strong circadian control. Circadian rhythmicity is generated by a network of clock genes, which are transcription factors capable of regulating the expression of other genes (Reppert and Weaver, 2002). Moreover, the SCN is strongly influenced by light entering the eye and, to a lesser extent, by other time cues such as temperature.

Homeostatic process (S) represents the sleep pressure and is sleep/wake dependent, so it increases as wakefulness progresses and decreases as sleep progresses. The level of Process S is therefore linked with previous wakefulness. Homeostatic control mechanisms are activated to contrast the excessive sleep loss or the prolonged sleep, in order to maintain a sort of "sleep equilibrium" (Paul Franken et al., 1998)

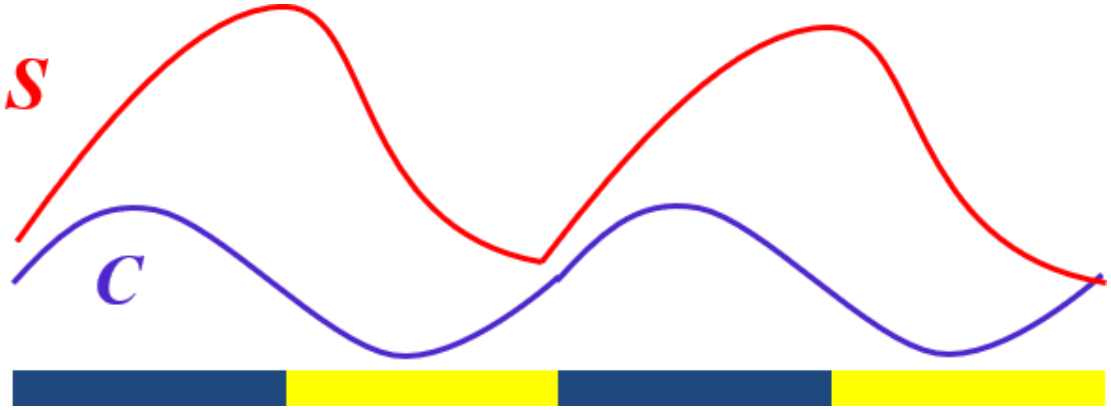


Figure 2 Sleep regulation processes. Homeostatic and circadian regulatory process in mice (nocturnal animals). Blue and yellow bands represent dark and light phase respectively.

Interaction between circadian and homeostatic processes in sleep regulation.

Circadian rhythms are generated by an oscillatory network of many transcription factors that give rise to a feedback loop. In mammals there are three main transcription factor taking part to this loop: CLOCK, NAPS2 and BMAL1. BMAL1 interacts with both CLOCK and NAPS2 forming heterodimers. The two dimers, BMAL1-CLOCK and BMAL1-NPAS2 drive transcription of Per1, Per2, Per3, Cry1, Cry2 genes. After entering the cell nucleus, the Per-Cry complex interrupts the transcription caused by the heterodimers (Figure 3).

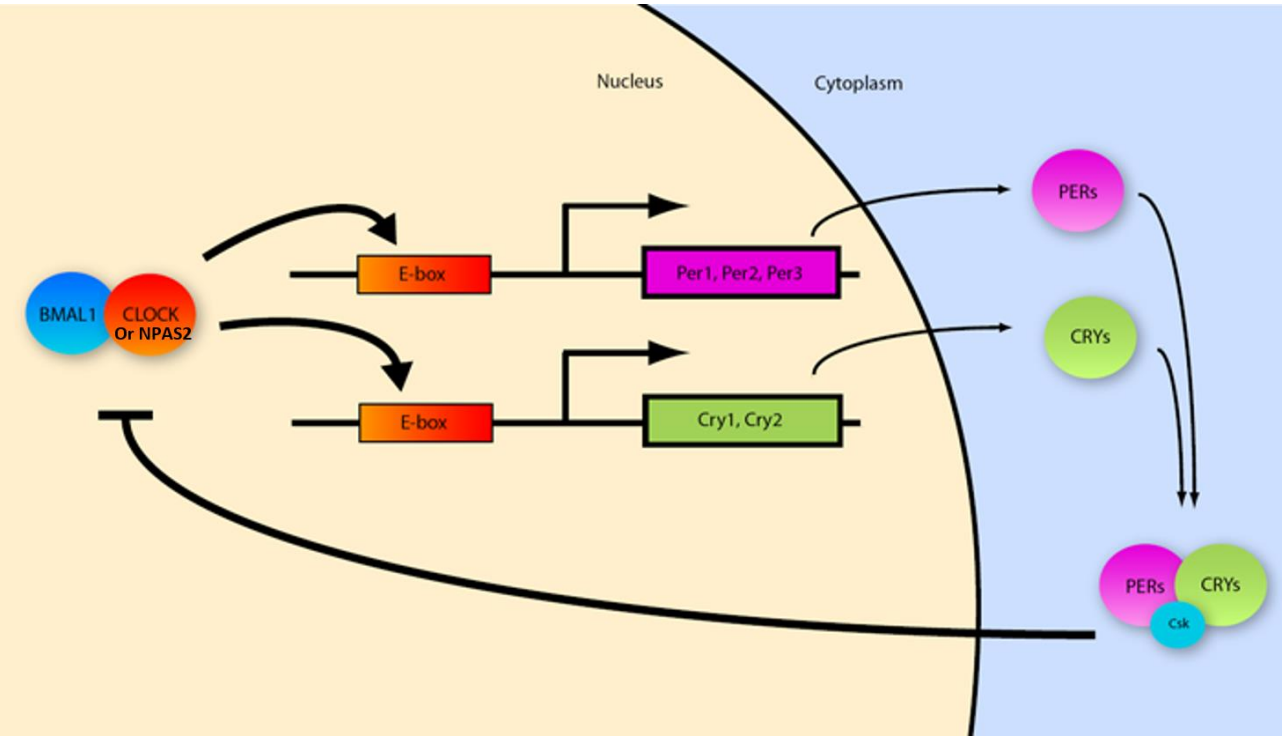


Figure 3. BMAL1-CLOCK/NPAS2 interaction. The heterodimers formation in the nucleus lead to the transcription of Per_s and Cry_s genes. They form complexes in the cytoplasm and then go back into the nucleus inhibiting BMAL1.

The importance of these genes on circadian rhythm has been demonstrated in several studies, in which it has been shown that knocking out a single of these genes or combining more than one involves the lack of circadian behavior (Takahashi et al., 2008).

Mice with a target disruption of clock genes, present also impairments in sleep homeostasis. Moreover, some of the genes above mentioned present expression linked to the time spent awake. Finally, the time spent awake affects the ability of BMAL1 to form heterodimers. All together these effects lead to a conclusion that also homeostatic response is under genetic regulation

(Franken, 2013). Some studies highlighted the effects of disruption of these genes in homeostatic features. In Cry1 and Cry2 KO mouse model was found an increased NREM sleep and EEG delta power associated with it (Wisor et al., 2002). Homozygous mice for BMAL1 deletion showed increase in total sleep time, sleep fragmentation and a reduce compensatory response to sleep deprivation (Laposky et al., 2005). In CLOCK mutant mice, decreased NREM sleep and NREM consolidation were observed together with a decreased compensatory mechanism for REM sleep (Naylor et al., 2000). Per1,2 double- mutant showed an increase in EEG delta power after sleep deprivation respect to wild type mice (Kopp et al., 2002; Shiromani et al., 2004). The increase in expression of the genes Per1 and Per2 depends on the strain considered. Franken and colleagues (Franken, P., and D-J. Dijk, 2009), have carried out a comparative study between different strains, where also the strains used as progenitors in the present study (AKR / J and DBA / 2J) were investigated. In particular, the results showed that the level of Per2 gene was significantly higher after 1hr, 3hr and 6hr after previous sleep deprivation in both strains AKR/J and DBA/2J. Moreover, the expression of Per1 and Per2 genes in the forebrain and SCN was found to follow the two distinct process: in SCN their expression followed the circadian rhythm while in forebrain was dependent on the sleep/wake distribution. Although, it has been thought that the two regulatory processes of sleep (C and S) were independent, however these results strongly support the hypothesis that these two mechanisms are interconnected each other in the regulation of sleep and wakefulness.

Epigenetics and role of genomic imprinting in sleep. Epigenetics can be defined as changes in gene functions that are heritable and that do not modify the DNA sequence. Among all the epigenetics mechanisms, one of the most interesting emerged in recent years is the genomic imprinting. Genomic imprinting is an epigenetic phenomenon by which certain genes are expressed in a parent-of-origin specific manner, that means that some phenotypes depends on the combination of the parental alleles. Usually, both copies of each gene are active, or silenced in cells. In some cases, only one of the two copies is normally expressed and the other is silenced. Which copy is active depends on the parent of origin: some genes are normally active only when they are inherited from the father; others are active only when inherited from the mother. Many imprinted genes influence embryonic, placental and neonatal growth, while others affect behavioral and neurological functions. Participation of genomic imprinting in so much different processes means that there's not a unique function among all the imprinted genes. There are several hypotheses addressed so far that may explain why the genomic imprinting is evolved in mammals. One of these hypothesis, proposed by Moore and Haig at the beginning of the 90's claims that imprinting has evolved in mammals because of the conflicting interests of maternal and paternal genes in relation to the transfer of nutrients from the mother to her offspring. Specifically, the imprinted genes expressed by the paternal allele promote embryonic growth maximizing the energies towards the offspring so as to promote the paternal genome. Conversely, the maternally expressed imprinted genes act to

suppress embryonic growth, so that maternal care can be equally distributed (Moore et al. 1991). This hypothesis may be explained by an opposite imprinting defects at chromosome 15q11–13, which are responsible for opposite sleep phenotypes as well as opposite neurodevelopmental abnormalities, namely the Prader-Willi syndrome (PWS) and the Angelman syndrome (AS). Specifically, PWS is due to loss of paternal expression of alleles, while the AS is due to loss of maternal expression gene. In the PWS, both human and mice (mice having the paternally deletion of the Snord116 gene) present alteration of sleep architecture, particularly related to REM sleep (Lassi et al., 2016). Additionally, excessive sleepiness and narcoleptic like symptoms were also observed (Vela-Bueno et al., 1984). Conversely, mice model of the AS (having the paternally deletion of the Ube3a Gene) show a reduction of both NREM and REM sleep (Ehlen 2015). In addition, this paper, in agreement with unpublished data came from our laboratory, performed on mice having the paternal deletion of the Snord116 gene, hypothesized that imprinted genes (Snord116 and Ube3A genes) may play an important role in the regulation of the homeostatic process of sleep (Pace et al, in preparation).

Perinatal environment, maternal cares and development. Parental cares influence cognitive and reproductive strategies development of the progeny. To assess the mechanism through which perinatal environment triggers cognitive development, many studies were conducted in rodents. Liu et colleagues (Liu et al., 2000) compared the maternal behavior in rats during the first ten post-natal days and evaluated the effect on hippocampal synaptogenesis and spatial learning and memory test. They found a significant higher spatial exploration in animals grown up with mothers that spent more time in licking and grooming behavior. Finally, they found a direct relation between maternal cares and hippocampal synaptogenesis. In other studies, variation in maternal cares and in particular in licking/grooming and arched-back nursing have been associated with certain forms of learning and memory. Bredy and colleagues (Bredy et al.,2003) showed that performance in memory tests, such as novel object recognition, are impaired in rat pups grown up with mothers spending less time in licking/grooming behavior. They also showed that enriched environment can reverse the effect, involving a sort of compensatory mechanism. Finally, it has been shown that maternal cares influence the development of neuronal systems implicated in the expression of fear; adult offspring of mothers investing more time in licking/grooming behavior and in arched/back nursing showed a reduce behavioral fearfulness respect to the offspring of mothers investing less time in that kind of behavior, even if the total amount of time spent with pups was similar (Caldji et al., 1998).

In humans, the formation of a secure attachment with the mother is a fundamental step for the progeny to develop appropriate reproductive strategy. Neonates acquire all the information about the surrounding environment from the caregivers. Therefore, if the child is able to develop a healthy attachment to the mother, it will be more likely to grow aware that the surrounding environment is favorable to a reproductive strategy that focuses on quality, rather than on the quantity of offspring. In this context, the adopted reproductive strategy will tend to delay sexual development at a suitable age and lengthen the interval between births (IBI). Conversely, if the infant / child grows up in a poor maternal environment, it is more likely that as an adult he develops a reproductive strategy that aims to maximize offspring by investing less time in caring for individual children (Belsky et al .,1991; Cassidy et al.,2000). This view is consistent with the hypothesis reported above, that genes of paternal origin in infants have been selected to increase the inter birth intervals (IBIs) in order to maximize maternal cares toward the progeny (David Haig., 2014) while the genes of maternal origin promote a shorter IBI in order to increase the survival rate of the progeny investing less energy and increasing the number of progeny (David Haig, 2014).

Moreover, the indifference on the part of the parents can lead the child to perceive the surrounding environment as hostile, choosing therefore to avoid the attachment to the mother, a more useful strategy in less favorable environments. The possible attachment patterns can be summarized in three

main categories: safe, avoiding and ambivalent. The secure attachment is the result of an adequate investment by parents in the care of children, the avoidant is caused by the reluctance of parents to take care of children and finally the ambivalent attachment is a sort of compensatory behavior adopted by children with parents unable to invest in their care (Chisholm et al., 1996). There seems to be a link between the reproductive strategy adopted by adults and REM sleep at a young age. Some studies have provided evidence that deprivation of REM sleep at a young age has an effect on sexual development later in life (Mirmiran et al., 1983). Furthermore, the abundance of REM sleep in the post-natal period in altricial species and its change in response to the mother's condition support a role of REM sleep in early development. A possible explanation of why REM sleep and not the NREM to be involved in this process may lies in the different pattern of brain activation in the two stages. During REM sleep there is a strong activation of the limbic areas and an almost total deactivation of the dorsolateral prefrontal cortex, while the NREM sleep is characterized by the activation of the temporal and parietal lobes and by the deactivation of the thalamus (Hofle et al. 1997). Evidences indicate that brain functions are influenced by the maternal and paternal genome through genomic imprinting. In particular, it seems that maternal genome influences functions of the brain areas activated during NREM sleep while paternal genome influences the development of the areas connected to REM sleep (Keverne et al.,1996). These results agree with the before mentioned conflict's theory (McNamara et al.,2002).

Sleep and sleep deprivation in cognitive processes

It has been already established, from studies performed both in humans and in animals, that slow waves (SW), which play an essential role in neuroplasticity and memory consolidation are linked to prefrontal cortex (PFC) functions. Prefrontal cortex (PFC) is implicated in a variety of cognitive processes in which top-down processing is needed and, in particular in goal-directed behavior such as decision-making. Moreover, it's strongly implicated in working-memory, the temporary memory of cognitive processes necessary for the task (Miller et al., 2001; Dalley et al., 2004) Different theories state that sleep has a restorative role increasing cognitive functions in subsequent wakefulness and that its specific stages are involved in modulation, regulation and also in the preparation of cognitive processes. Moreover, sleep seems to be involved in different memory processes (Walker et al., 2009). In particular, in both humans and animals, declarative memory is more sensitive to NREM sleep deprivation while role of REM sleep is more linked to procedural memory (Kyriacou et al., 2010). Role of sleep in cognition is influenced by circadian clocks and cognitive impairments in circadian instability are usually associated with sleep deprivation. There are different hypothesis on the mechanisms trough which sleep deprivation affects cognitive processes. It has been proposed that sleep deprivation affects cognitive processes mediated through prefrontal cortex (PFC) , affecting high level of cognitive functions, such as decision making, stimuli processing and attention (Harrison & Horne, 2000; Ratcliff et al.,2009; Durmer & Dinges,

2005; Doran, Van Dongen, & Dinges, 2001). In fact, PFC lesions lead to different cognitive deficits, including attention problems, meaning that PFC mediated the maintenance of the attention towards external stimuli related to cognitive tasks also in presence of confounding factors. Also spatial orientation, shot-term memory and associative learning are affected by lesioning PFC.

Aims of project

Parent-of-origin effects play an important role in the regulation and modulation of different phenotypes such as sleep-wake cycle, homeostatic response and development.

The main purpose of this project was to investigate the role of parent-of-origin effects on different phenotypic expressions. To investigate the parent-of-origin the reciprocals crosses of inbred strains mice were used. The progeny would be heterozygous and phenotypic differences would be due to the parental background.

In addition, to understand whether parent-of-origin may interfere with the expression of some physiological behaviors we assessed in these mice the sleep-wake cycle, some behavioral tests (i.e cognitive performance in complex task, long term memory task and social behavior) and neuronal activity.

Sleep-wake Cycle was investigated in adults reciprocal cohorts, already used in a previous study conducted in our laboratory (Tinarelli et al. 2014), and the EEG/EMG profiles were analyzed to evaluate the role of parent-of-origin effects on sleep architecture. Animals were kept in foster condition with CD1 female to highlight the importance of the genetic background and eliminate the variable of maternal cares. We studied the sleep architecture of the experimental groups in baseline condition and then we measured the homeostatic response to six hours of total sleep deprivation. Then, since it has been shown that sleep alterations affect cognitive performance (Alhola et al., 2007), we tested our animals in typical time-based decision-making task (Switch Task), in which animals had to focus on the duration of a light signal and respond to it correctly (see Material and Methods). Also in this case, the experiment was conducted before and after total sleep deprivation. Finally, we assessed the spontaneous activity of a cerebral area highly involved in both sleep and cognition, such as Prefrontal Cortex (PFC) in baseline condition and immediately after sleep deprivation.

Perinatal environment and maternal cares were investigated to determine how these features can influence different kind of behavior in age of development, in particular the attachment behavior, novel object recognition and social interaction

First of all, we investigated reproductive strategies of mouse strains used as parental background (AKR/J and DBA/2J, see Material and Methods for further details). Then we tested both progenitors and progenies in three main

behavioral domains: mother-pups bonding, long term memory, through novel object recognition test and finally on social behavior. In this second part of the project, reciprocal crosses were divided into different subgroups: foster condition and biological mother. In the first case, as for the first part, pups were moved to a foster mother (CD1) at birth, while in the second condition, the biological mother grown up the pups. To assess mother-pups bonding, we used an already published test (Lassi. G, Tucci. V.,2017) in which the puppies are made to interact with another adult mouse (same sex and age) and the curiosity and ability to interact with others in the presence and absence of the mother is measured. This experiment was conducted on P18, before weaning when the puppies are still dependent on their mother's care.

The long-term memory and the curiosity for novel objects of the same animals was tested at a more mature age (5-7 weeks of age) through the novel object recognition test with a 24-hour retention period. Finally, social behavior and curiosity towards new subjects was measured using a social interaction test in which the animals were left free to explore different unknown animals.

All the procedures and experimental designs used int his project will be explained in the Materials and Methods section, and subsequently I'll describe main results obtained. Last part of the thesis will be about conclusions and discussion.

Materials and methods

Animals

Reciprocal crosses of mouse inbred strains DBA/2J and AKR/J (F1: AKR/J x DBA/2J, F1r: DBA/2J x AKR/J, mother always reported first) were used (Figure 4). Animals were kept in home cages in controlled temperature conditions (22 ± 1 °C) with 12L: 12D light-dark cycle, with light phase starting at 8:00. Water and food were available ad libitum except for behavioral experiment. All animal procedures were approved by the Animal Research Committee and the Veterinary Office of Italy, for Istituto Italiano di Tecnologia (IIT) Genova.

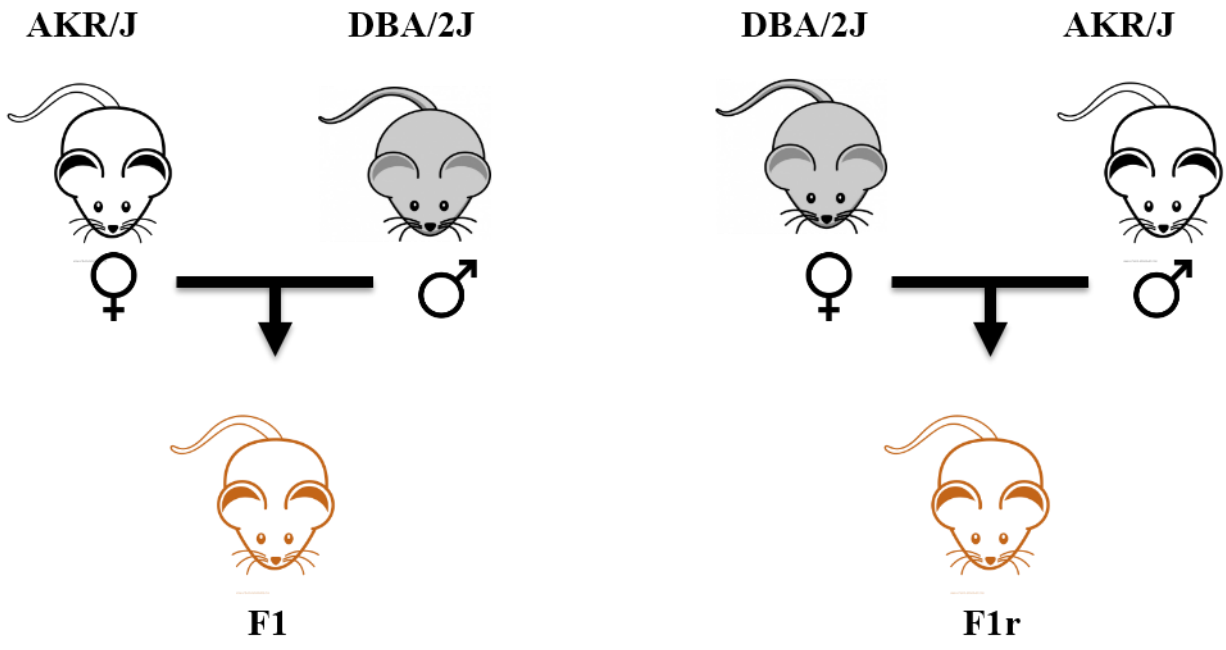


Figure 4. Experimental groups. Reciprocal crosses of AKR/J and DBA/2J inbred strains were used

Sleep Study

Surgery and data acquisition

F1 (n=8) and F1r (n=6) animals in foster condition with CD1 female were used. The electroencephalogram combined with electromyogram (EEG/EMG) were used to assess the changes in the sleep-wake cycle across the two strains of mice. Mice were anesthetized using 1.5%–2.5% isoflurane in oxygen and surgically implanted with a telemetric transmitter (volume, 1.9cm 3; total weight, 3.9g; TL11M2-F20-EET; DSI, St. Paul, MN, USA) connected to electrodes for continuous EEG/EMG recordings for the assessment of the sleep-wake cycle. A wireless EEGs transmitter/receiver, was subcutaneously implanted. EEGs wire electrodes were implanted epidurally in the right hemisphere in the frontal cortex (coordinates: 2 mm posterior of the bregma and 2 mm lateral to the midline in the right parietal skull) and parietal cortex (coordinates: 3 mm anterior of the lambda and 2 mm lateral to the midline in the right frontal skull). EMG was recorded by 2 stainless steel wires inserted bilaterally into the neck muscles ~5mm apart and sutured in place.

Following surgery, all animals received paracetamol (200 mg/kg; once a day; PO; Tempra) and enrofloxacine (10mg/kg; once a day; SC; Baytril) for two days after surgery. Animals were housed individually in their home cages for a recovery period of 7 days, and then each mouse was recorded for the EEG/EMG continuously for 24h for a baseline value (BL) and for the following 18h after perturbing sleep by 6h of sleep deprivation (RC).

Cortical EEG/EMG signals were recorded using Dataquest A.R.T. (Data Science International). Signals were digitized at 500 Hz sampling rate with cut-off frequency at 50 Hz. EEG signals were low pass filtered at 0.3 Hz and high pass filtered at 0.1 KHz respectively. The polysomnographic recordings were visually scored offline using SleepSign software (Kissei Comtec Co. Ltd, Japan), 4s epoch window, identifying wakefulness (W), NREM or REM sleep stages as previously described.

Experimental protocol and scoring

Sleep-wake cycle was recorded in baseline condition for 24h to assess the normal sleep architecture of the experimental groups. The day after, from the beginning of the light phase (8:00), animals were sleep deprived for 6h by gentle handling, in order to reduce the stress. Immediately after the sleep deprivation, we recorded additional 18h of recovery sleep, in order to evaluate the homeostatic response to sleep deprivation. (Figure 5).

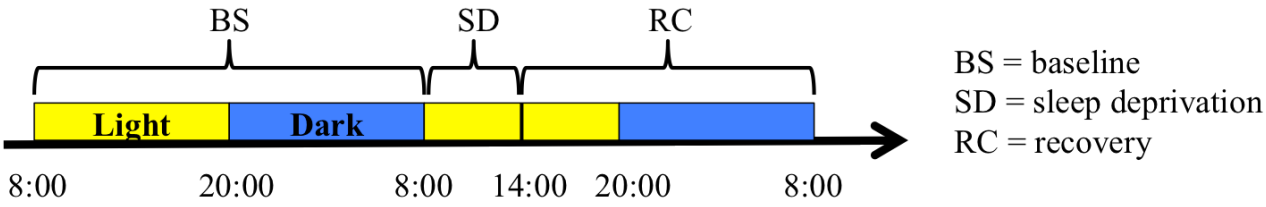


Figure 5. Experimental design sleep

Data were scored offline at the end of the 48 hours of recording. Data were imported into Sleep Sign software to detect sleep stages and extract power spectra. W, NREM, and REM states were scored when characteristic

EEG/EMG activity occupied 75% of the epoch. The amount of sleep was calculated for NREM and REM sleep. Through the Fast Fourier transform, we calculated the power in delta (0.25-5 Hz) and theta (5-9 Hz) frequency ranges, typical of NREM and REM respectively. Classification of sleep and wake stages was performed based on the following criteria:

REM sleep (paradoxical sleep) is characterized by EEG oscillation in the theta range (5-9 Hz) and muscle atonia (almost flat EMG)

NREM sleep is characterized by slow oscillations in EEG signal (delta range 0.25-5 Hz) and reduced EMG signal

WAKE is characterized by high frequencies oscillations in EEG signal and intense EMG signal

EEG and EMD signal were visually scored and when the signal was unclear, affected by artefact movement or electrical noise, the epochs (4s) were tagged and excluded from subsequent analyses.

Sleep data analysis

Sleep data were analyzed by using Phenopy (Balzani et al.2018), Python and MATLAB. To extract sleep architecture features, the time course of NREM and REM sleep was calculated. Time course was expressed as a percentage of time spent in a specific stage. Data were analyzed in two hours bins and plotted as mean ± SEM (Figure 12 A-B). Sleep rebound, defined as the difference between time spent in REM and NREM sleep stages during the first two hours of recovery and the two hours during the same time of the day

of baseline was expressed as bar graph representing mean ± SEM (Figure 12 C). We calculated the power associated to the characteristic band of frequency for NREM and REM sleep. Power was extracted with the Fast Fourier Transform of the EEG signal for the sleep stage and band of frequencies of interest (Figure 12 D-E). Finally, to assess the distribution of power along the entire range of frequencies (0-20 Hz) during the recovery phase, we extracted the power density in that range for REM sleep (Figure 12 F). Data are shown as mean ± SEM.

Behavioral study - switch task

Apparatus. Animals were tested in home-cages system as was recently described by our group (Maggi et al. 2014, Balzani et al. 2018). In each cage was placed a COWE (Cognition and Welfare, TSE Systems, Germany), an operant wall equipped with 3 hoppers able to detect nose-poking activity through infrared beams. Inside each hopper was placed also a LED for luminous stimulation. Each home cage was placed inside a box insulated from light and noise and equipped with an air recirculation fan and a programmable house light according to the desired light-dark cycle. In this experiment the light was programmed to keep the animals in 12L: 12D condition with the light turned on at 8:00. In this experiment F1 (n=10) and F1r (n=10) mice at 16-20 weeks of age were used. During the experiment welfare and body weight of the animals were monitored, to maintain animals at 90% of initial

weight. The COWE was remotely controlled via a computer (TSE Systems-OBS software).

Procedure. Switch task (Balci et al. 2008) implies the association between a fixed duration of light stimulus with one of the two lateral hoppers. Experimental design was composed of a pre-training phase and two training phases after few days of habituation in the home-cages system. In the first days of habituation 30 dustless precision pellet were put in the cage. During the pretraining phase animals learned to associate nose poking in the central hopper with food delivery in the two lateral ones. Animals could self-start trials in all experimental phases by poking into the central hopper, then, during the training phases, had to decide to nose poke in one of the two lateral hoppers in response to a light signal to receive the reward (Figure 6).

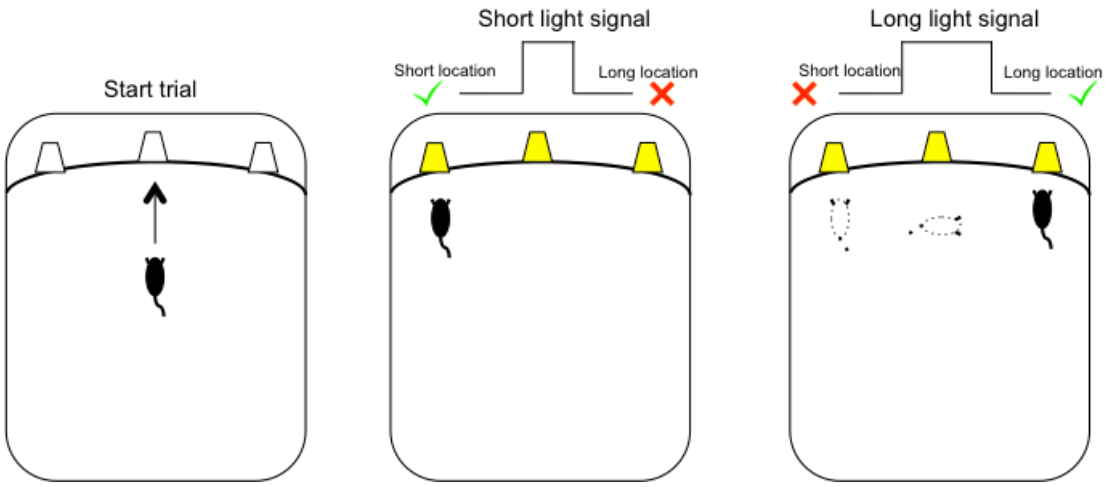


Figure 6. **Experimental design switch task**

Pretraining. During the pretraining phase, that lasted for 3-5 days, mice started the trial by poking into the central hopper. LED into lateral hoppers turned on and mice were able to poke into the lateral hoppers to receive the food reward. Trials duration was fixed at 30 s. When infrared beam inside one of the lateral hoppers was broken the LED inside it turned off and a food pellet was delivered; in this phase the order of pokes was irrelevant. Between one trial and the next one there was an inter-trial interval (ITI) composed by a fixed duration (30 sec) plus an additional random delay calculated as geometric distribution around the mean of 60 sec.

Training. During the training phase mice had to start the trial poking into the central hopper and lights into the lateral ones turned on. During this phase one fixed duration of the light was associated with one of the two hoppers for food delivery. During the first training phase the ratio between lights duration was maintained to 3:9: one hopper was associated with 3 sec light duration (short) and the other one with 9 sec (long) light duration. Type of trial was automatically random determined with the same probability (50%). Animals had to estimate the duration of the light and respond accordingly. A trial was considered correct if the first answer given at the end of the light stimulus was in the correct hopper. After few days of training animals learned the association between light duration and food location. Usually mice started to poke into the short location and then, if the lights stayed on the switched to the long one. Moving too soon or too late entailed the end of trial without reward. A percentage (20%) of trials without reward also in case of correct

answer were added. In order to avoid that results were influenced by location preference, experimental groups were divided in two subgroups: for half of them, short signal was associated to left hopper and long duration with right one, for the other group was the opposite. When animals reached the steady state in this first training phase, usually after 5-7 days, the ratio between light signals was decreased to 1:2. In this case short signal lasted for 3 sec and the long one for 6 sec. When the steady state was reached in this second paradigm, animals were sleep deprived at the beginning of the light phase, as described above, and then tested again for another week.

Behavioral data analysis

We recorded every action performed by the animal within the home cage, data were acquired by using PhenoScale software and exported and analyzed with tools developed in Python and MATLAB environment. Although every action from mice were recorded, however only data from the last training phase and from long trials were analyzed.

Error rate was calculated as the amount of wrong trials among total number of rewarded trials and expressed in percentage. Data were analyzed in one-hour bins at the baseline, over the recovery period and after recovery from previous SD. Inner bar graphs represent the error rate for light and dark phase. Data are presented as mean ± SEM (Figure 13 A). Secondly, to analyze performance of each animal we plotted the relative time of switch compared

to its coefficient of variation (CV) related to the optimal switch time, mathematically calculated (Figure 13 B). Finally, the cumulative switch latency was extracted. Bar plots inside graphs represent time accuracy (TA) calculated as the median ($Q_{0,5}$) and time precision (TP) calculated as the difference between third and first interquartile ($Q_{0,75}$-$Q_{0,25}$) (Figure 13 C).

Electrophysiological study – Single unit activity recordings

Surgery

Under deep isoflurane anesthesia (1.5-2% volume in oxygen) and sterile conditions, mice (F1=4 and F1r=5 grown up with CD1 foster mother) 16-18 weeks old at time of surgery, were implanted with a 16 electrodes matrix (Tucker Davis Technology) in medial Prefrontal Cortex (mPFC). Animals were fixed to stereotaxic apparatus and a subcutaneous injection of local painkiller (Lidocaine) was practiced before proceeding with surgery; then the skull was exposed, and a small craniotomy (2mm) was performed, dura mater was removed for placement of the matrix. Three screws were positioned as far as possible from the craniotomy and one of those was used to fix the grounding wire. Remaining two screws were used to help dental acrylic to fix to the skull. Electrodes were then drive in position (from bregma: AP =1.9, ML=0.3, DV=-2.5 mm) with an electronic micro positioner connected to the stereotaxic. When the electrodes reached the final location, the two-component silicon gel (World Precision Instruments, FL, USA) was used to

seal the craniotomy and protect the surface of the brain from dental acrylic. After the time necessary to harden the gel (about 10 minutes) dental acrylic was used to fix the array to the skull. At the end of the surgery a dose of antibiotic (Baytril-Bayer) was administered to prevent infections and the animals were made to wake up from anesthesia under a heat lamp in order to check their state of health before moving them into single cages. Before the start of the recording phase we waited at least one week to let animals recover from surgery.

Experimental design and data acquisition

Experiment was conducted into a behaving box designed for this purpose (AM-Microsystems) in which animals were free to move. To assess differences in the activity of the mPFC in response to sleep perturbation, we recorded 2 hours of spontaneous activity during the light phase of day 1 in which animals were free to sleep then, the day after, at the beginning the light phase mice were sleep deprived (SD) for 6h as described before. At the end of the sleep deprivation we recorded other 2 hours of spontaneous activity during the recovery phase. Also in this phase animals were free to sleep (Figure 7).

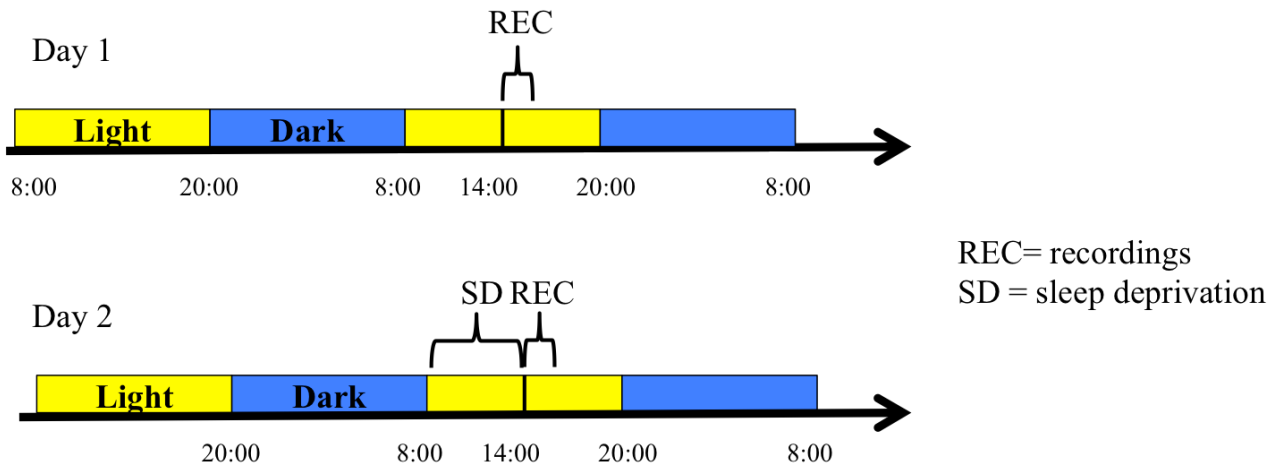


Figure 7. **Experimental protocol single unit activity recordings**

Single unit activity (SUA) was recorded with MAP acquisition system (Plexon Inc). Signals were sampled at 40 kHz. Through filter settings, neural traces were initially filtered with a band bass filter (300 - 5000 Hz). Spike sorting was made offline with OfflineSorter (Plexon Inc) using a semi-automatic Expectation-Maximization algorithm. Data analysis was made using ad-hoc prepared MATLAB scripts. In order to be sure that we were comparing the activity of the same cell in different conditions, only cells with a similar waveform in baseline and recovery were considered for the analysis (Figure 8).

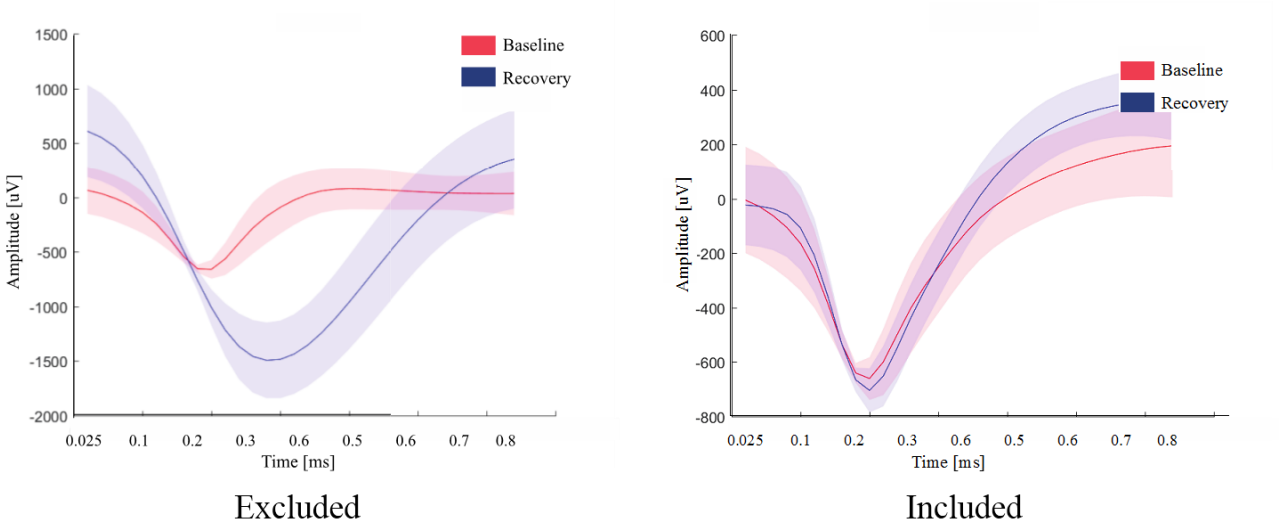


Figure 8. **Waveforms selection**

Activity changing between baseline and recovery was assessed considering the mean activity in the two phases and cells were labeled as "Up" if the mean activity of recovery phase was higher that the arbitrary threshold of 10% respect to baseline activity, while cells with a mean activity in recovery lower than baseline activity, at least of 10% were labeled as "Down". Remaining cells were classified as "Equal". Putative excitatory and inhibitory neurons were identified applying the Jitter method (Fujisawa et al. 2008) on each spike train (Jitter window [-5:5 ms]) and then analyzing the cross correlograms (CC) (Figure 9).

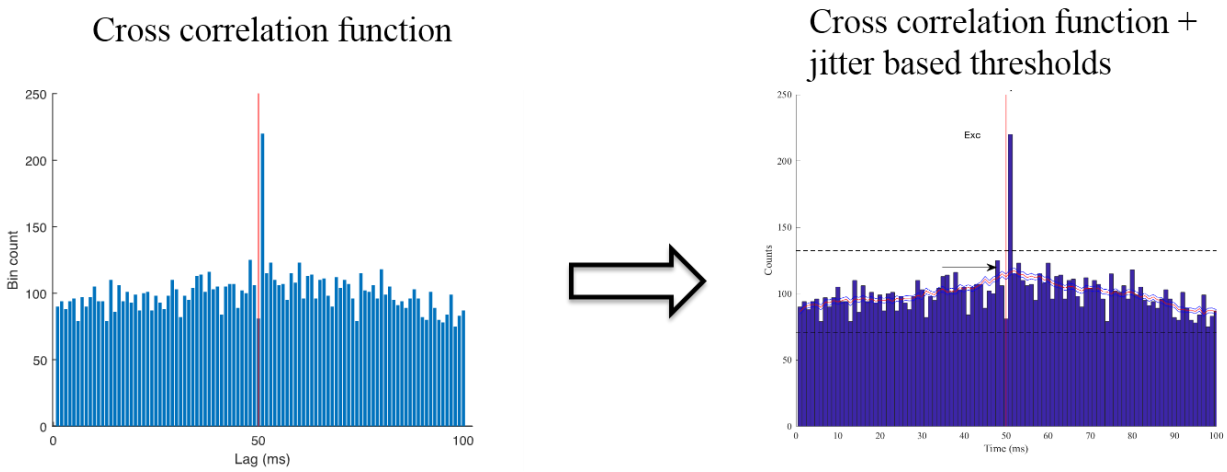


Figure 9. **Jitter based method applied to cross correlograms.** Left panel shows the cross correlogram between to neurons, the right panel show the same cross correlogram with the threshold obtained from the algorithm

If the peak between 0-4ms was higher that global maximum, the connection was labeled as putative excitatory, if the peak was lower than the global minimum, connection was labeled as putative inhibitory; otherwise, two neurons were considered as unconnected (Figure 10). In this phase we considered as reference cells only those that showed a mean firing rate higher that 1Hz.

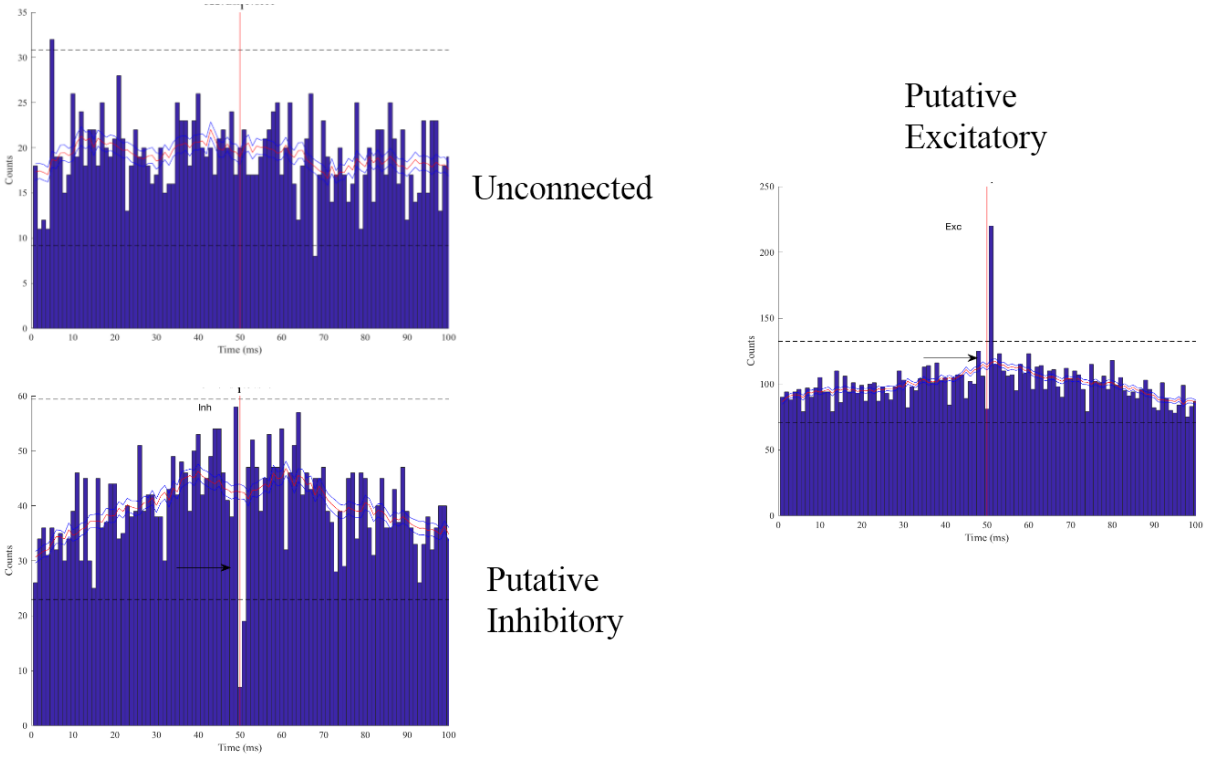


Figure 10. **Synaptic connections analysis**

Histology. Array position was verified through histological procedure. Mice were deeply anesthetized and a small current (5 mA, 10sec) was passed through the probe. Then the animals were sacrified and trans-cardially perfused with parafolmaldehyde solution. The brains were sectioned on a freezing microtome at 40µm in the coronal plate. Slices were mounted on slides, Nissl-stained and cover-slipped to be checked at the microscope (Figure 11).

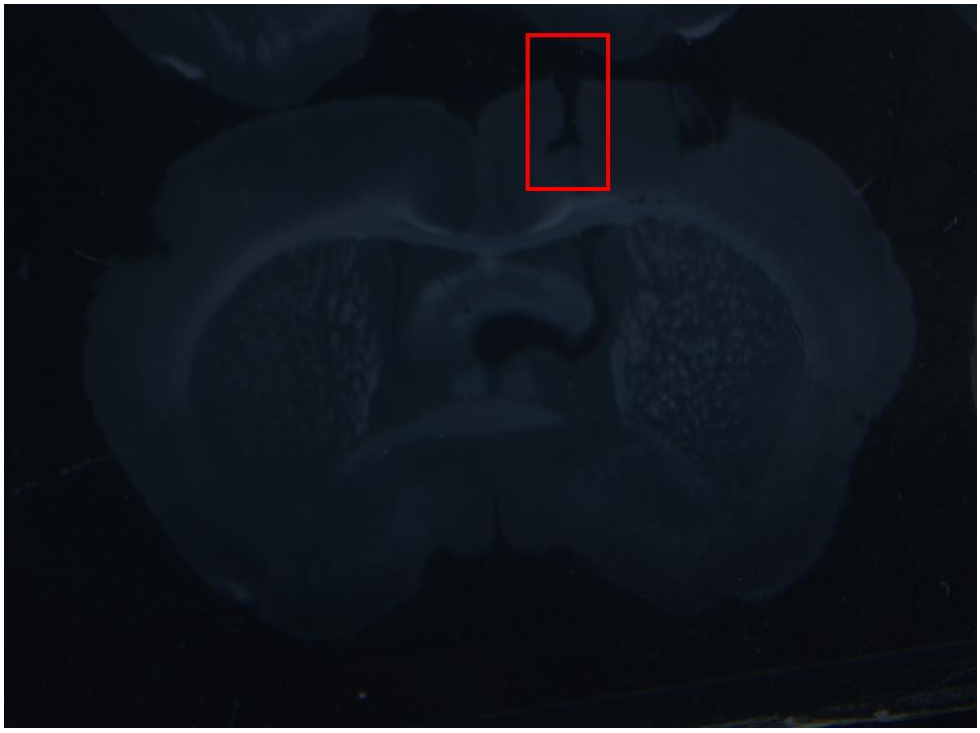


Figure 11. **Implant position**

Perinatal features extraction

To assess wheter the progenitors of the experimental groups adopt different reproductive strategies, some variables of our animals (DBA/2J and AKR/J) were extracted from the information system for tracking the colonies of our institute (PyRAT). Since each animal was tracked throughout its life, was possible to extract different variables. We considered a sample of females crossed with males of the same strain and with male of the second strain: DBA/2JxDBA/2J (n=20), AKR/JxAKR/J (n=31), DBA/2JxAKR/J (n=18) and AKR/JxDBA/2J (n=17). Inter births interval was extracted as difference

between birth dates, while and percentage of failed pregnancies was considered as the number of females in permanent breeding without births among all considered females (Figure 18,19). Reproductive strategies are strictly linked to the environment, so these data are meaningful only considering our animal facility.

Mouse Strange Situation Procedure (MSSP)

Apparatus and procedure

F1_bio(n=17), F1r_bio (n=16), F1_fost (n=21), F1r_fost(n=7), AKR/J (n=11) and DBA/2J (n=12) were used. Two litters for each group were tested at P18 to assess mother-pup bond in biological condition and in foster condition, in which puppies are removed from the biological mother and entrusted to an adoptive mother CD1. Experiments was conducted in a grey arena (60x40x40 cm) in which animals were free to move. Test was designed to assess the mother-pup bonding in three consecutive trials of 3 minutes preceded by an habituation phase of 15 minutes in which the mother was left in the arena with a virgin age-matched CD1 female to familiarize.

During the first trial the pup is placed in the arena with the mother and the stranger. During the second trial the mother was removed and the pup was left alone with the stranger. Finally, in the third trial mother was inserted again into the box (Figure 12).

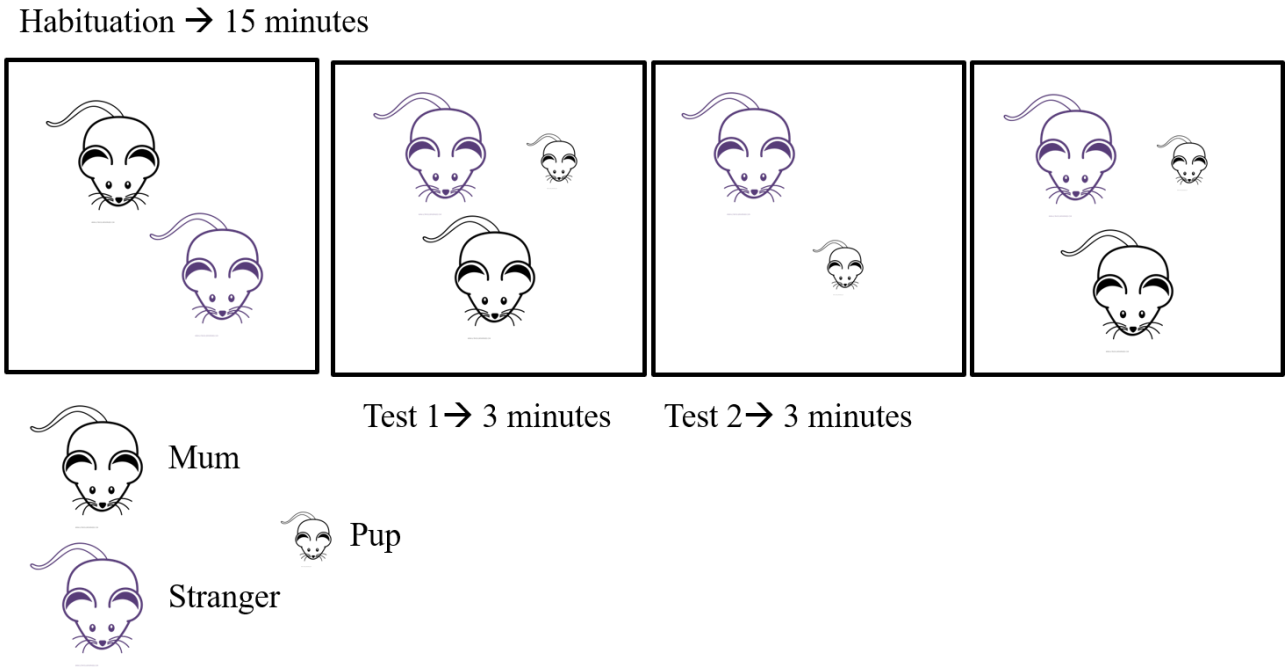


Figure 12. **Experimental protocol MSSP**

Data analysis. We identified three behavioral response: "maternal preference ", in which pups prefer the mother to the stranger, "reunion" defined as an increased exploration of the mother in the last episodes respect to the first episode and "stranger effect" characterized by an intensive exploration of the stranger during the second episode respect to others. The experiments were video recorded with a webcam and scored manually offline. Data are presented as mean ± SEM (Figure 20,21,22).

Novel Object Recognition (NOR)

Apparatus and procedure

Experiment was conducted in a transparent arena (40x40x35 cm) in which animals were free to move. Same animals tested in the MSSPs were used between 5-7 weeks of age. We habituated each animal to the arena for 10 minutes. The day after animals were put into the arena in presence of two identical objects (Lego blocks or flasks, black or white) for 10 minutes. After 24h of retention period, animals were tested with a copy of the previous object, to avoid any olfactory residues, and a new object (complementary shape and color) for 10 minutes (Figure 13).

Data analysis. We measure the time spent exploring the two identical objects during the day 1 and time exploring the new object compare to the old one during day 2 and, to evaluate the ability of the animals to discriminate between old and new object, we measured the difference score, as difference between exploration time of the new object respect to know one, and the discrimination index, as percentage of time spent exploring the new object respect to the old one. Data are presented as mean ± SEM (Figure 23, 24,25,26). The experiments were video recorded with a webcam and scored manually offline.

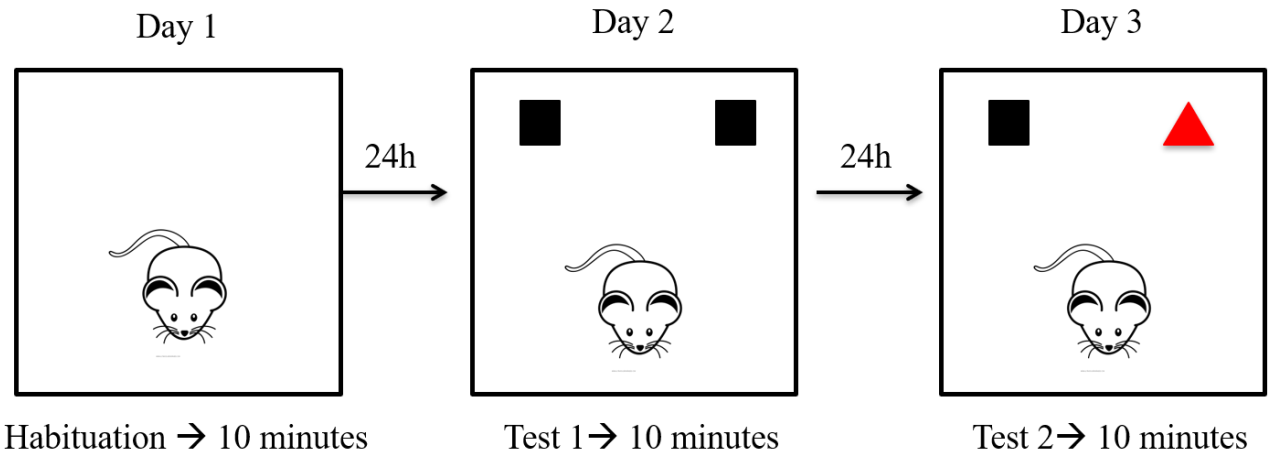


Figure 13. **Experimental protocol NOR**

Social Interaction Test (SIT)

Apparatus and procedure

Experiment was conducted in a gray arena (60x40x40 cm) in which animals were free to move.

Same animals tested in the MSSP an NOR were used between 7-9 weeks of age. We habituated each animal to the arena for 5 minutes. The experiment was designed by five 1-minute trials interspersed with 3-minute inter-trial interval (ITI). During the first 4 trials the same stranger CD1, same age and sex as the experimental mouse, was inserted into the arena. During the last trial the stranger was replaced with another, never met by the experimental mouse (Figure 14).

To avoid high levels of stress among the animals used as strangers, particular attention has been paid to rotating the animals, so as not to subject the same to too many consecutive trials.

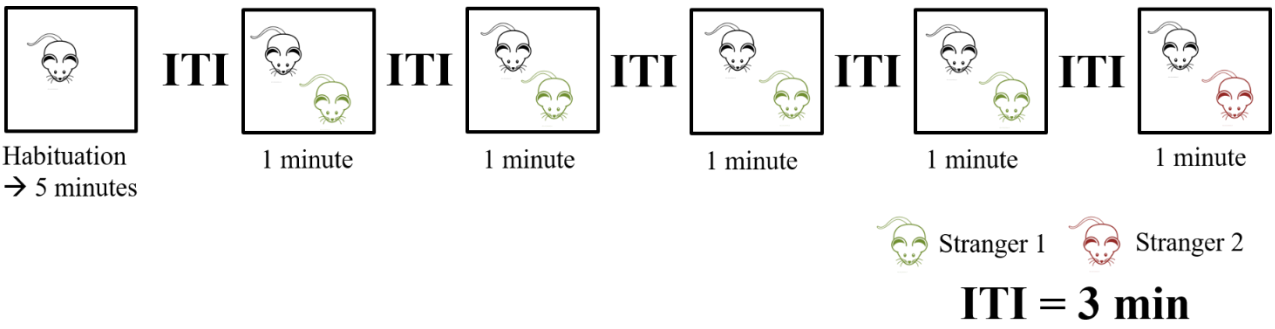


Figure 14. **Experimental protocol SIT**

Data analysis. We measure the time spent the first strangers across the first four trials respect to the time spent exploring the new one in the last trial. Sniffing, licking and general exploring of the other animal were considered as social events and measured, fights between the animals or attempts of the other animals avoided were not considered as social event. Each experiment was video recorded with a webcam and scored manually offline. Data are presented as mean ± SEM (Figure 27).

Results

Sleep Results

To assess if the two experimental groups presented differences in sleep architecture and the homeostatic process of sleep, daily sleep profile were investigated at the baseline (BL) and 18h after a period of SD, which represents the recovery period (RC). Our results show that at the BL during the light phase, the two groups presented a similar architecture in both sleep stages, NREM and REM sleep (Figure 15A-B). However, during the last four hours of the dark phase, F1 spent more time in both NREM and REM sleep stages compare to F1r group (Figure 15 A-B). Although the F1r group spent less time in NREM sleep during the last four hours of the dark period, however, the EEG delta power of NREM was significantly increased at this time relative to F1 group (Figure 15 D). No differences were observed for the EEG theta power in REM sleep between the two groups of mice (Figure 15 E). Interestingly, in the RC period, accounting for the 6h after previous SD, the amount of time spent in NREM sleep was unchanged between the two strains of mice, while REM sleep was increased in the in the F1r group during the first hours from 2h to 4h of the RC period relative to F1 group (Figure 15 A, B). Sleep rebound was calculated as the difference between time spent in NREM and REM sleep during the early two hours of recovery (first bin) and the same time of the day during baseline (ZT 6-7). Our data shows that while F1r group increased the time spent in both REM and NREM sleep immediately after sleep deprivation, in F1 group only REM sleep is increased

in response to sleep deprivation, while the amount of NREM sleep decreased during the first hours of recovery (Figure 12 C). The EEG delta power of NREM sleep which represents a marker for the homeostatic response to sleep was unchanged between the two groups during the first 6h of the RC time during the light period. During the dark period, the F1r group showed a significant increase in the delta power compared to the F1 group (Figure 15 D). Regarding the EEG theta power of REM sleep, as observed at the BL no changes were displayed over the RC time, in both dark and light periods. However, when we analyzed the power densities of REM sleep the F1 group didn't show changes concerning the mean value of the RC (black dashed line), while in the F1r group the theta power decreased immediately after the SD. With the proceeding of RC, the power density clearly showed the difference in the temporal distribution, in fact, during the first two and six hours of the RC time, the theta power of the F1 group decreased again respect to baseline mean, while in the F1r increased nearing the values observed during the baseline. Overall, these results suggest that although in the F1 group the amount of NREM sleep was increased in the BL only during the dark period, which represents the active phase for mice, the NREM power was reduced compared to F1r group. This reduction in the NREM power was also observed in the RC period after sleep deprivation but not immediately after the perturbation but 6h after. In addition, in the RC time, even a reduction in the power densities of REM sleep was observed.

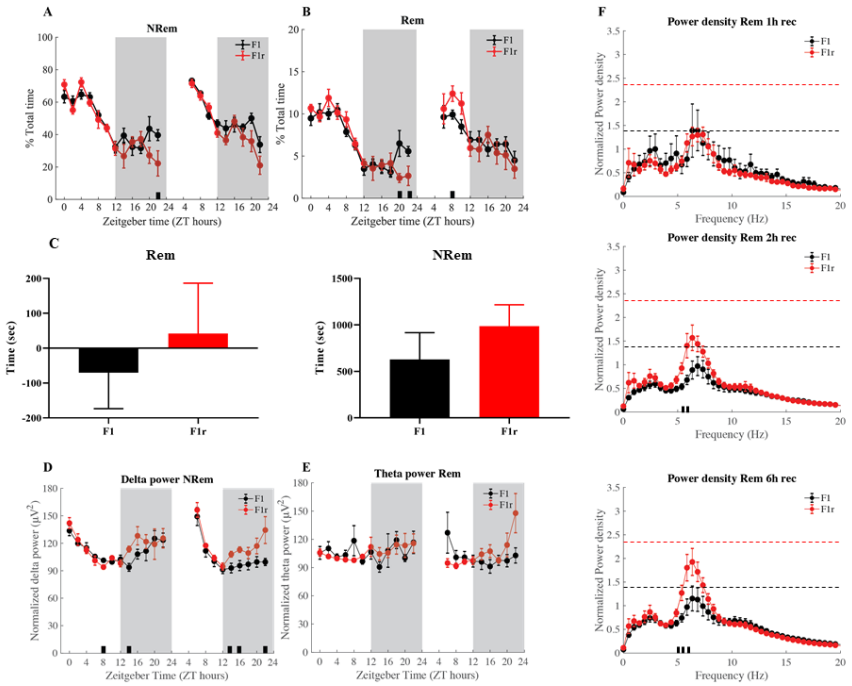


Figure 15. **Sleep results** A-B) Time course NREM and REM sleep. X-axis represents Zeitgeber time in which ZT0 coincides with the beginning of the light phase. Grey areas represent dark phases. Each time point represents 2-hours bin. C) Sleep rebound, calculated as the difference of time spent in REM and NREM stages during the first 2-hours of recovery and the two hours of baseline corresponding to the same time of the day. D-E) Delta power of NREM sleep and theta power of REM sleep. Statistical significance calculated through two-way ANOVA. F) Power densities of REM sleep along different time points of recovery phase. Black boxes in the X-axis represent statistical significance calculated through one-way ANOVA ($p<0.05$).

Behavioral results – switch task

To assess differences in the experimental groups in the cognitive performance before and after SD, we tested our animals in a typical time-based decision-making task (see Methods). Results were divided into three different phases: baseline (BS), recovery (RC) representing the first 18 hours after sleep deprivation and after recovery (AR), starting from the end of recovery till the end of the experiment (1 week after SD). The first measure extracted was the error rate along different experimental phases. Our results didn't show differences in the error rate during the light phase of BS between the two strains of mice, while during the dark period of the same experimental phase, the F1 group showed a higher error rate compared to the F1r group (Figure 16 A, left panel). The error rate was increased in both light and dark phase of RC, as expected in both groups compared to the previous BL (Figure 16 A, middle panel.). Interestingly the F1 group presented a significantly higher error rate even after the recovery phase, both in light and dark period (Figure 16 A, right panel) compared to F1r group. Figure 16 B shows the optimality surface for the three stages representing the individual performance regarding the time of the switch, related to its coefficient of variation (CV). Our results showed that during baseline and after recovery phase performance of each animal is around the optimal time of switch (red line). Only during the recovery phase, the timing strategy adopted from the two experimental group is different, showing the higher coefficient of variation in F1r group and the switch time of some animal is further from the optimal line. To observe the

timing strategy as mean value for group, we plotted the cumulative switch latency (Figure 16 C) that shows that the two groups adopt the same timing strategy during the baseline, in which the two curves are superimposed, and there are no differences in time accuracy (TA) and time precision (TP) (inner box plots). During the recovery phase, the cumulative curve shows the different timing strategy of the two groups while box plots show that, even if the average values are similar for both TA and TP, the performance of F1r group are more spread respect to F1. Finally, after recovery phase timing strategy returned to baseline condition and our data don't show differences between groups. Overall these results indicate that sleep deprivation significantly affects the F1 group, which also presented a significant increase in the error rate from the BL. Interestingly F1 group did not change the timing strategy adopted in response to SD as conversely observed for the F1r group.

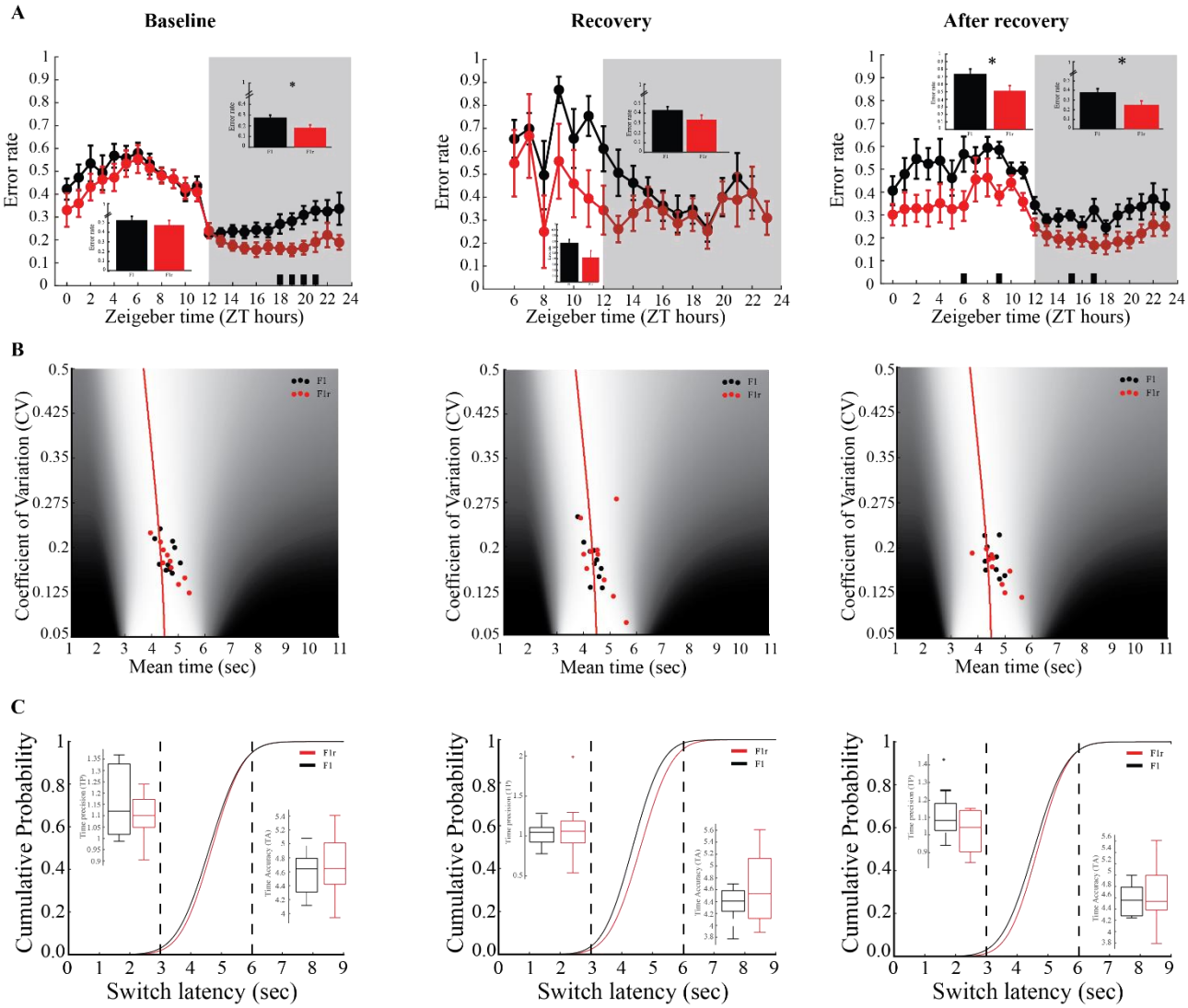


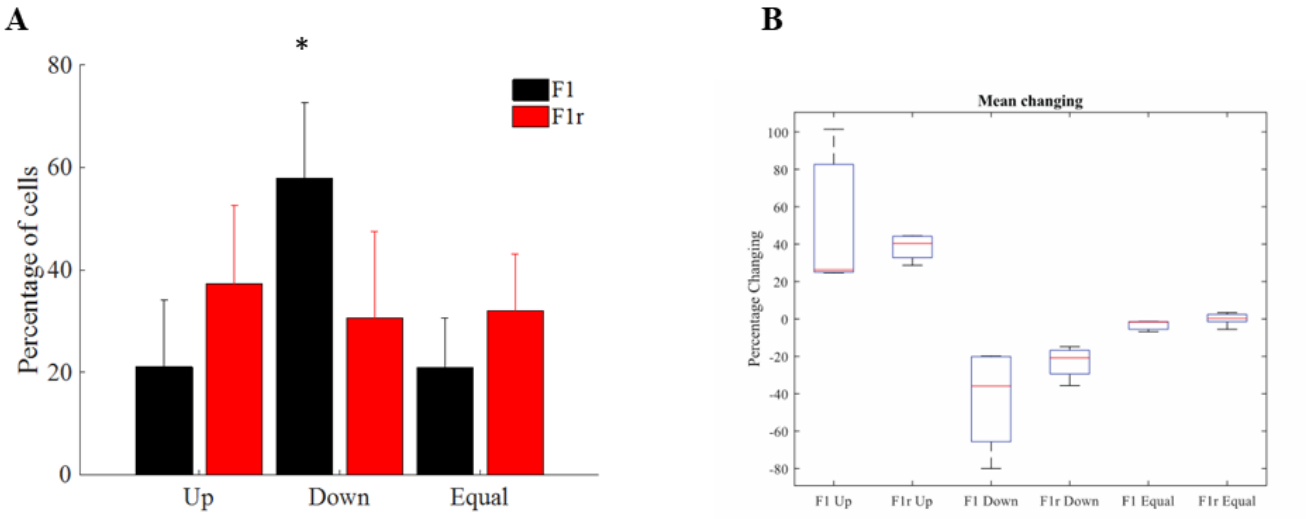
Figure 16. Switch Results A) Error rate during baseline (left panel), recovery (middle panel) and after recovery phases (right panel). X-axis shows Zeitgeber time and Y-axis shows the error rate as percentage. Grey areas represent dark phases. Inner bar graphs represent the mean error rate for light and dark phases expressed as mean ± SEM. Black boxes in the X-axis represent statistical significance (P<0,05). B) Optimality surface for each experimental phase. X- axis represents the mean time of switch in seconds, Y-axis represents coefficient of Variation (CV) and the red line represents the optimal time of switch (optimality curve). Each dot is a different animal. C) Cumulative distribution of switch time for each experimental phase. X-axis represents the switch time in seconds and the Y-axis represents the

cumulative probability of switch. Dashed lines represent the light signal durations, 3 and 6 seconds (see Materials and Methods). Inner box plots represent time accuracy (TA) and time precision (TP).

Electrophysiological results – Single unit activity recordings

PFC is one of the brain areas most involved in the correct execution of complex cognitive tasks. We analyzed the spontaneous activity of mPFC neurons before and after sleep deprivation. Cell classification was based on the mean firing rate during recovery respect to baseline normalized with an arbitrary threshold (see Material and Methods). This analysis showed that that majority of F1 recorded cells respond to sleep deprivation with a decrease in their activity (Figure 17 A) and a higher variability in the percentage of changing of firing rate (Figure 17 B). To assess synaptic connections between recorded cells, we applied a Jitter-based algorithm to cross correlograms (see Material and Methods). During the baseline recording, high connectivity was observed as expected in both groups investigated, since the cerebral area is involved in different processes, with a majority of putative excitatory connections relative to the putative inhibitory connections (Figure 17 C). In response to sleep deprivation, the percentage of F1 connected cells increased and in particular, the number of putative excitatory connections was significantly higher relative to F1r. Conversely, for the F1r group, any differences were observed regarding the number of connections as well as the distribution of the excitatory and inhibitory connections. The more significant amount of connections can be due to a change in cells firing rate, that allowed

the inclusion of more cells in the Jitter algorithm or the formation of a new synaptic connection in response to sleep deprivation.



C

%	F1 baseline	F1 recovery	F1r baseline	F1r recovery
Connected	75.09	92.34	74.34	73.89
Excitatory	40.21	48.67	42.37	43.865
Inhibitory	4.39	4.01	6.16	5.56

F1, n = 73
F1r, n = 121

Figure 17 Electrophysiological results. A) Percentage of cells for each category (Up, Down, Equal). Asterisks represent statistical significance calculated from Chi-Square test ($p<0.05$). B) Mean percentage of changing in the activity for each category. C) Synaptic connections results. Percentage of connected cells and relative percentage of putative excitatory and inhibitory connections during baseline and recovery phase. Total number of recorded cells is reported for each group.

Perinatal features

Interbirth interval analysis (Figure 18) shows that DBA/2J females crossed with either DBA/2J males or AKR/J males have an interbirth interval higher than AKR/J females. Reciprocal crosses of both inbred strains reduce the percentage of failed pregnancies respect to inbred crosses (Figure 19). Those results suggest that the inbred strains that we used as parental background present a different reproductive strategy.

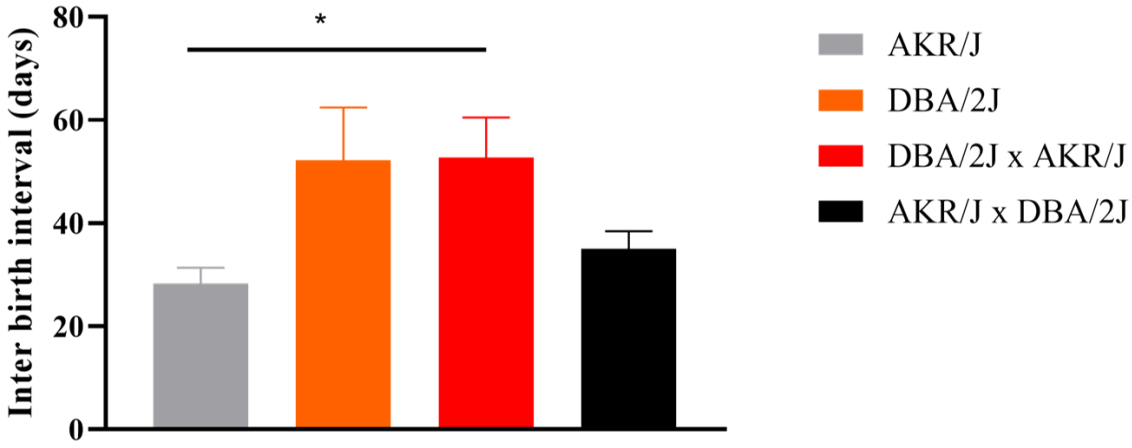


Figure 18 **Interbirth interval**. Interbirth interval for females of each group. Data are presented as mean ± SEM and asterisks indicate statistical significance, calculated through one-way ANOVA with Tuckey's post hoc for multiple comparisons, *$p<0.05$.

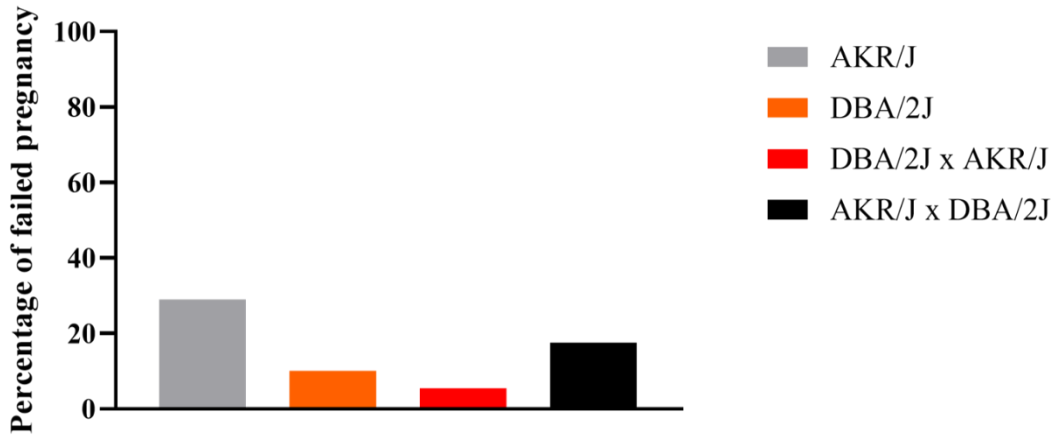


Figure 19. Percentage of failed pregnancy. Percentage of failed pregnancy for each group of breeding.

MSSP Results

Each trial was analyzed separately. We measured the "maternal preference," represented as the comparison between time spent with the mother and the stranger during the last test (Figure 20). Our results show that DBA/2J pups did not present maternal preference behavior, in fact, they spent the same amount of time in exploring the mother and the stranger. The same effect was present in (AKR/J x DBA/2J, F1r) grown up with biological mother, while the same group in foster condition spent less time exploring the mother respect to the stranger. In AKR/J pups and reciprocal groups from the same mother both biological and foster condition present maternal preference

behavior. These results suggest that the foster condition makes more evident the phenotype due to parent-of-origin effect.

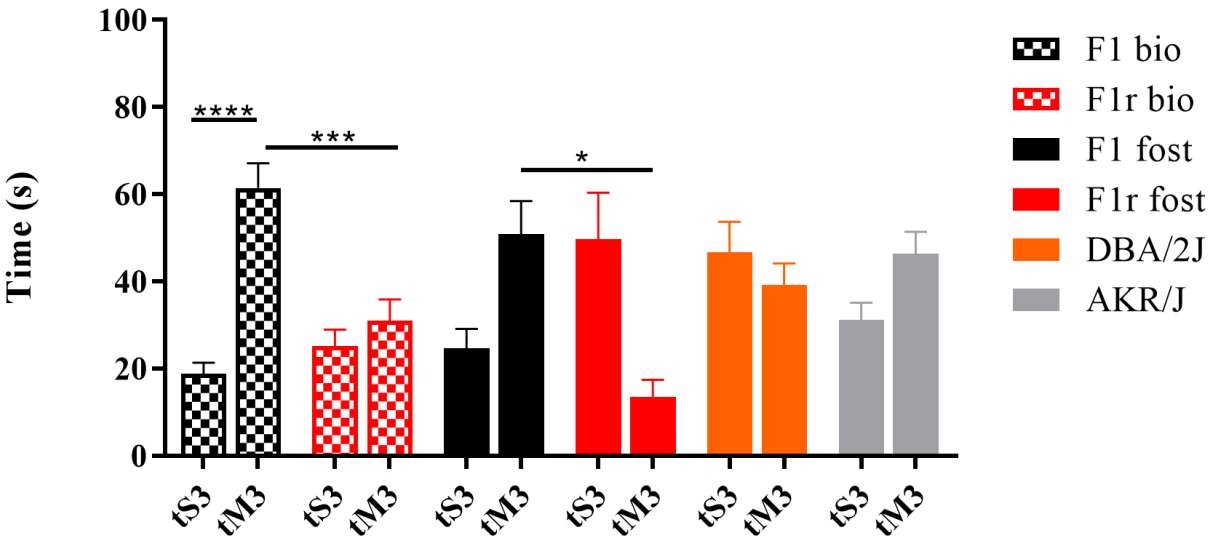


Figure 20 **Maternal preference**. Time spent exploring the mother and the stranger during the last trial. Statistical significance was assessed with two-way ANOVA with Tukey's multiple comparison test,*p<0,05,**p<0.01,***p<0.001,****p<0.0005.

We measured the effect of the reintegration of the mother in the last trial as the comparison between time spent by pups exploring the mother during the first previous test. DBA/2J puppies and reciprocals obtained from the same strain as a mother, presented less interest in the mother studying respect to other groups; in this case, as shown in Figure 20, foster condition for DBA/2J x AKR/J (F1r) crosses highlights the phenotype (Figure 21).

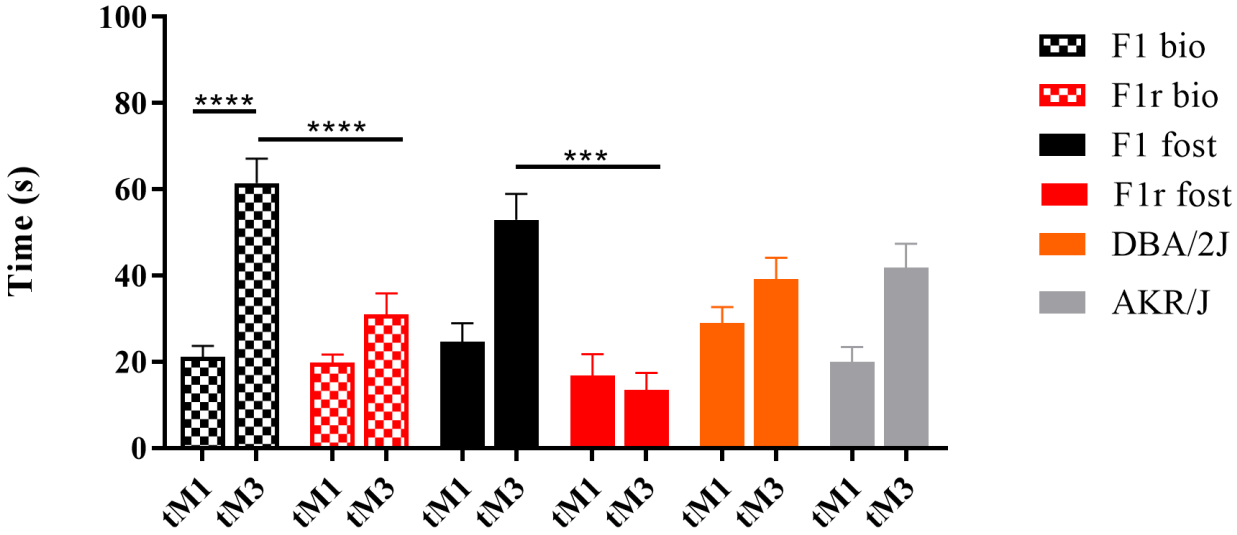


Figure 21 **Reunion**. Time spent exploring the mother during the first and the last trial for each group. Statistical significance was assessed with two-way ANOVA with Tukey's multiple comparison test ,*p<0,05,**p<0.01,***p<0.001,****p<0.0005.

Finally, we measured the stranger effect in terms of time spent exploring the stranger mouse during all the trials (Figure 22). All experimental groups, except for DBA/2J x AKR/J (F1r) in foster condition, showed an increase in exploration time of the stranger mouse during the second trial, when the mother was absent and decrease during the last test in which the mother come back (see Materials and Methods). F1r mice in foster condition showed the same exploration time of the stranger during the second and the third trial.

Overall these results suggest that foster condition plays a role in highlighting or masking some phenotypic traits, but mother-pup bonding is mainly under parent-of-origin effects.

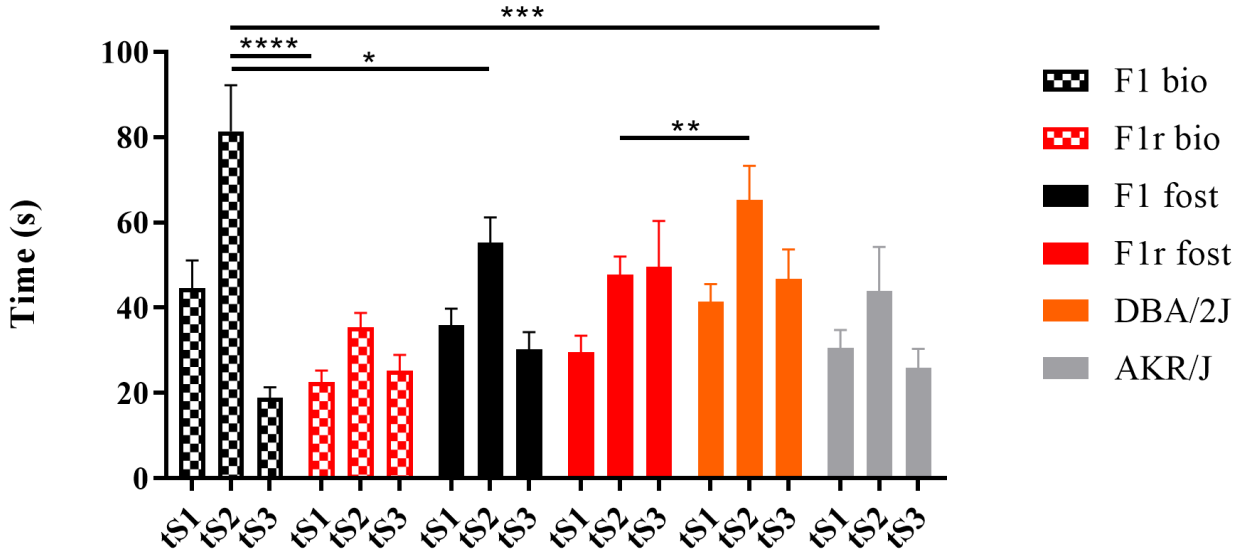


Figure 22 **Stranger effect.** Time spent exploring the stranger mouse during all trials. Statistical significance was assessed with two-way ANOVA with Tukey's multiple comparison test ,*p<0,05,**p<0.01,***p<0.001,****p<0.0005.

NOR Results

Each trial was analyzed separately for the two experimental days were mice were able to explore the objects. During the training day (day 1) animals are presented with two identical objects in shape and color (see Material and Methods) and we could not find any differences between strains of mice (Figure 23). The day after, in which one of the two objects is replaced with a new one with different color and shape, both groups of mice spent more time for recognized the objects, and no statistically significant difference was observed between groups of mice (Figure 24).

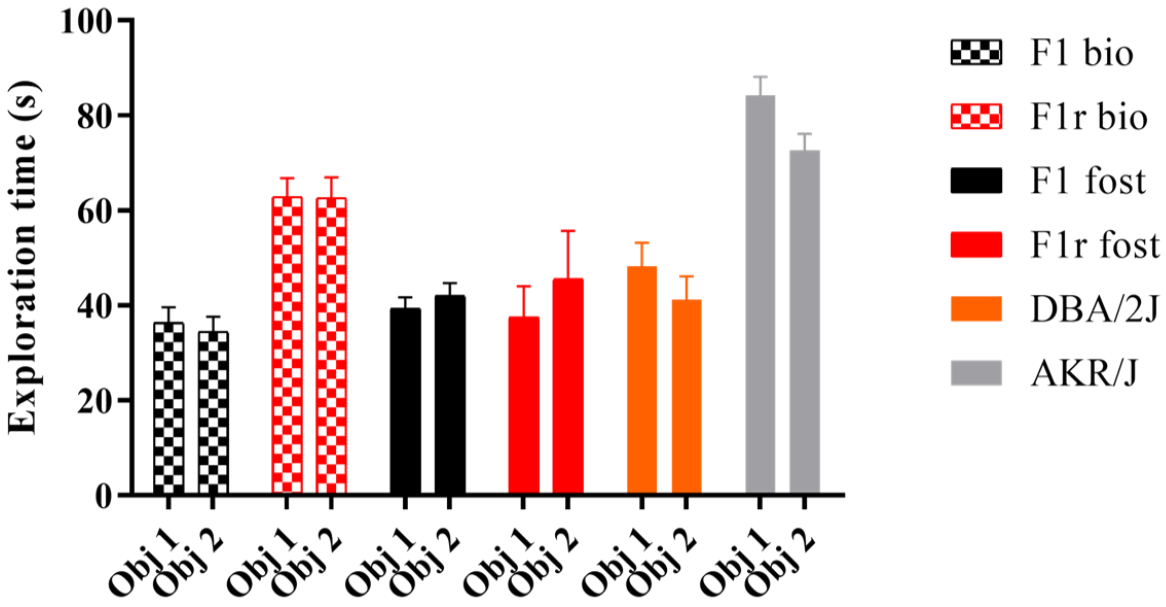


Figure 23 Training day. Exploration time of the two identical objects during the training day.

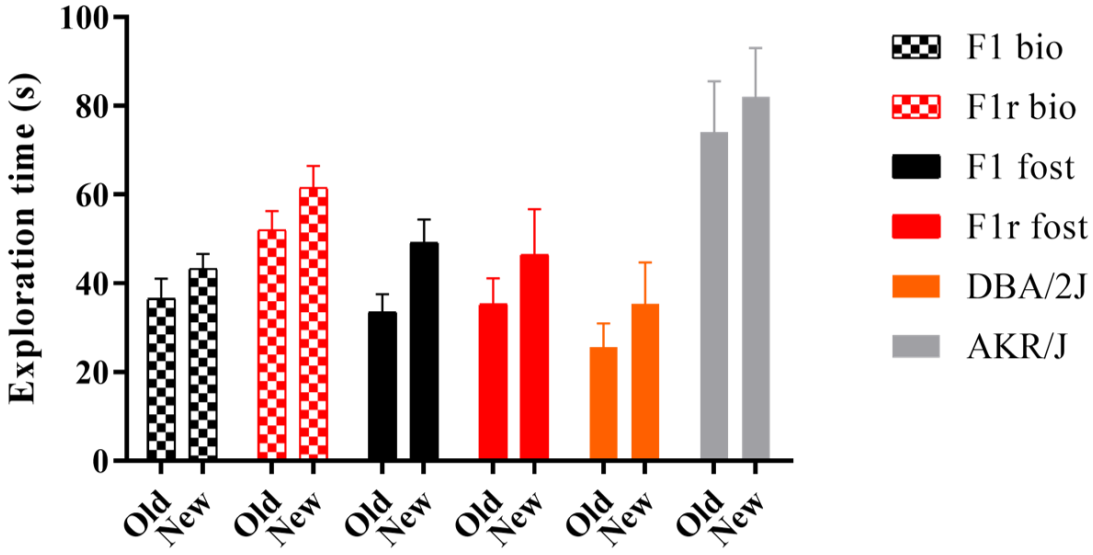


Figure 24. Testing day. Exploration time of the new object respect to the old one during the testing day

To assess the ability to discriminate between the two objects we measured the discrimination index (see Material and Methods). Our results show that all the experimental groups have a discrimination index around 50 %, indicating that the ability to discriminate between old and new object was poor (Figure 25).

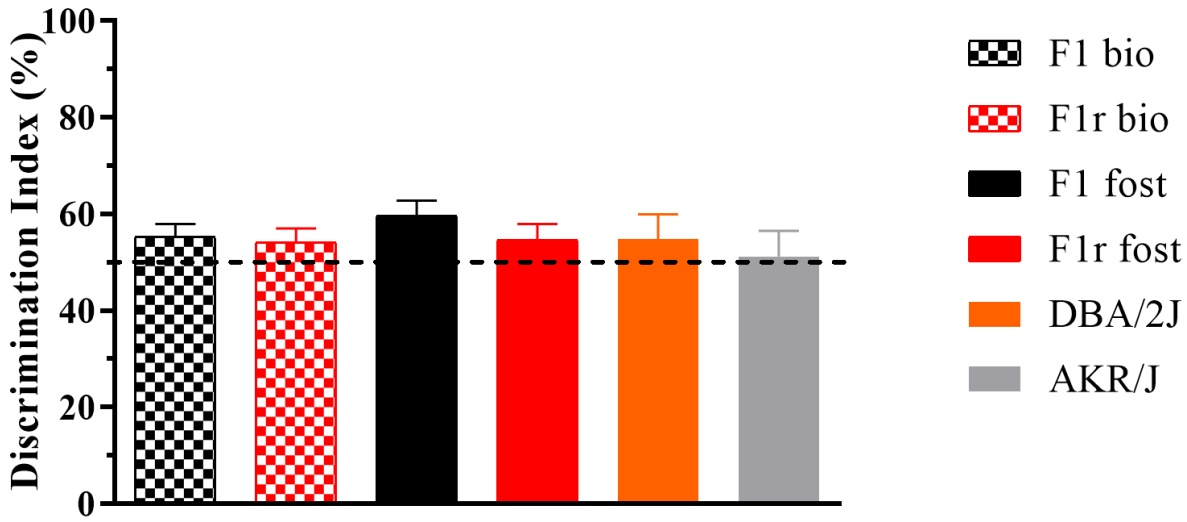


Figure 25 Discrimination index. Discrimination index of all experimental groups

Finally, we calculated the difference score, to assess if ,even not significant, the exploration time of the new object was higher than the old one. Our data show great variability between groups and inside each group, as shown by the high SEM. The mean value of each group is low, confirming that they explore equally the old object and the new one (Figure 26).

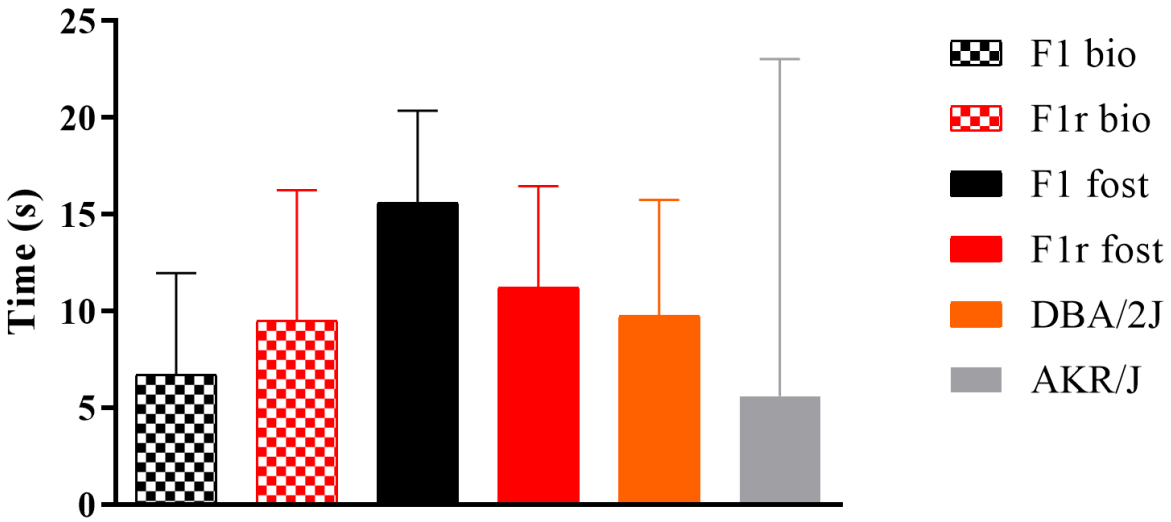


Figure 26 Difference score. Difference score for each experimental group, calculated as the difference between the exploration time of the new object compared to the old one.

SIT Results

Social interaction test (SIT) is a simple behavioral task that allows to investigate sociability of the animals and in some way, their anxious state. Mice are curious animals, and their attitude leads them to explore the new environment and other mice presented in it. We analyzed each of the five 1-minute trials separately, and we calculated the time spent in social behavior. Usually, the curiosity towards the first stranger decreases with the progress of the trials, and when a new subject is presented, in the last trial, a peak of interest is observed again, expressed as a time of exploration towards the other.

Our results show that reciprocal groups, both those in fostering and those in biological mother spent significantly less time with the stranger after the first trial. The curiosity resumed during the last trial in which the stranger is

different. In the case of F1r in fostering, the difference is not significant at the statistical level but indicates a trend in behavior. Both progenitors showed less curiosity towards strangers respect to other groups. Furthermore, they did not seem to discriminate between different trials (Figure 27).

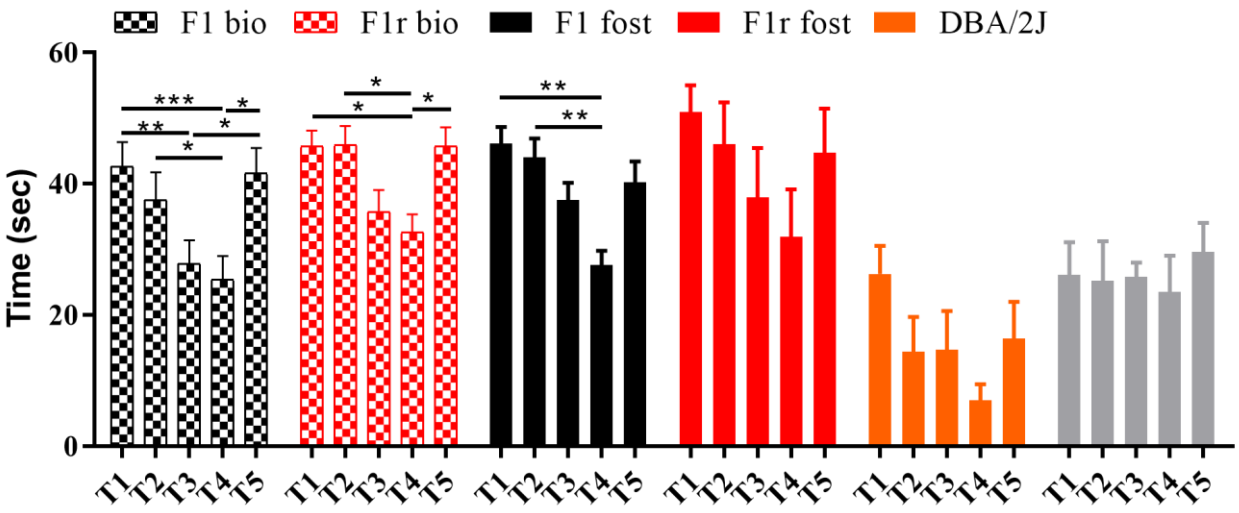


Figure 27 Social interaction test. Time spent exploring first stranger animal (T1-T4) and the new one (T5). Statistical significance was assessed with two-way ANOVA with Tukey's multiple comparison test,*p<0,05,**p<0.01,***p<0.001,****p<0.0005.

Discussion

In recent years, the role of parental background in the definition of different complex phenotypes has been made evident by numerous studies (Lawson et al.,2013). Some of these, have used the reciprocal crosses to highlight the role of target genes, both imprinted and not, showing that it is a promising experimental approach (Mott et al., 2014). Genomic imprinting and, in a broader sense, the parent-of-origin effects, take place from conception and many of the imprinted genes analyzed up to now, both in humans and rodents, have an effect on pre- and post-natal development and on some complex traits such as the structure of sleep and cognitive processes. Furthermore, a strong link between parent-of-origin effects, sleep architecture and perinatal environment has been suggested. Parental cares have been demonstrated to affect cognitive development of the progeny.

The main purpose of the first part of this work was to determine how parent-of-origin effects can affect complex phenotypes in adulthood. To answer to our first scientific question, we used reciprocal crosses as experimental approach, often used to determine the role of a target gene on complex traits. We have chosen as progenitors two inbred strains of mice widely studied in relation to sleep architecture in baseline condition and to the homeostatic response to sleep deprivation. Franken and colleagues demonstrated that EEG patterns during sleep and wakefulness and homeostatic response to sleep deprivation depend on the considered strain (Franken et al.,1998; Franken et al.,1999; Franken et al., 2001). Our results show that during the light phase

of baseline condition reciprocal crosses do not present differences neither in the amount of time spent in both NREM and REM sleep, nor in the corresponding delta and theta powers. During the dark period of the same phase we found that an increase in the amount of NREM (F1) is preceded by a decrease in the delta power and vice versa. Regarding the homeostatic response following sleep deprivation, our results did not show differences between the two groups in terms of time spent in NREM sleep and in the delta power associated with it. Furthermore, for both groups, both variables do not deviate from the values observed during the baseline. During the first hours following the sleep deprivation we observed an increase in the amount of REM in the F1r, and a corresponding decrease in the theta power associated with it. Since the F1 begin to sleep during the active phase, this is reflected in a lower need for sleep as a result of sleep deprivation (Figure 12 C) . Our data seems to confirm data reported in literature (Franken et al.,1998) about an inverse relation between amount of both NREM and REM sleep and the relative powers. Despite the fact that there were no differences in theta power neither in baseline nor in recovery, it is interesting that the peak profile of theta frequencies during the first 6 hours following sleep deprivation is significantly different in the two experimental groups and follow different timing profile. Similar results were observed in the progenitors during the baseline. In particular, AKR/J show a higher peak in theta frequencies than DBA/2J. When we analyzed performance of our animals in a time based decision making task (Switch task, see Materials and Methods), we didn't

find differences neither in the error rate during the light phase of the baseline nor in the overall timing strategy adopted by the experimental groups, but we found that F1 group shows a greater error rate during the active phase of the baseline. In the 18h following sleep deprivation, we found, as expected, an increase in the error rate in both groups, but only F1r group changed its timing strategy in response to sleep perturbation. Interestingly, at the end of the recovery period (After recovery, see Behavioral results), F1 showed a significant higher error rate respect to F1r in both light and dark phase. Some studies have highlighted a potential role of theta hippocampal oscillations in learning and memory processes. In rats, theta oscillations are also present during exploratory behaviors, as well as in REM sleep (Buzsaki et al., 1990) and it has been hypothesized that during the theta oscillations observed in exploratory behaviors, the newly acquired information are temporarily stored in hippocampus and subsequently converted into long-term memory (Buzsaki, 1989). Although our recordings have occurred at the cortical level (EEG) our data suggest a possible link between behavioral performances after 18h recovery in F1 and the different temporal profile of theta power following sleep deprivation. Finally, was already established that prefrontal cortex is particularly sensitive to excessive wakefulness (Muzur et al.,2002). Our results show that in F1 group the majority of recorded cells respond to sleep perturbation with a decrease in their activity and, in the meantime, a synaptic reorganization, as well as new synaptic formation may be triggered by sleep deprivation in the same group.

Our results confirm that some aspects of the response to sleep deprivation are due to parent-of-origin effects. The same strains of animals were already tested in the homeostatic response to sleep deprivation in a previous work from our laboratory (Tinarelli et al., 2014) that confirmed that genetic expression in response to sleep deprivation depends on parental background and, looking at other results reported in literature (Franken, P., and D-J. Dijk, 2009), it seems to be paternally derived.

In the second part of this work we were interested in assessing the importance of the perinatal environment and maternal cares in cognitive development of the progeny. To do this we assessed the differences in reproductive strategy of progenitors. Our results show that the strategies adopted from the females used as progenitors are different and, in particular DBA/2J females, both in inbred and outbred crosses present a longer inter birth interval respect to AKR/J females (Figure 18). As expected from the literature (Keller et al.,2002), outbred crossings decrease the percentage of failed pregnancy (Figure 19). To test whether these results have an effect on maternal care and if these affect some basic behaviors, we have tested offspring in attachment to the mother, in the novel object recognition test and in social behavior during development (see Materials and Methods). Our results show that both reciprocal crosses in biological conditions present “maternal preference” behavior and the same effect was observed in AKR/J pups, even if the difference between tS3 and tM3 is significant only for F1 animals. In fostering condition F1 animals (AKR/J x DBA/2J) maintain the maternal

preference, while in F1r animals the effect is lost, and pups showed avoidance attachment behavior, as happened in DBA/2J pups. All experimental groups, except for F1r in foster condition showed an increase in time spent with the mother during the last phase of the test respect to the first one (see Materials and Methods). When we compared the time spent exploring the stranger along different trials, all of the experimental groups, again except for F1r animals in foster condition showed the "stranger effect" behavior. All together our results suggest a difference in maternal cares between DBA/2J and AKR/J females. Progeny of the former showed a less secure attachment compared to the latter progeny. Moreover, in our results, foster condition highlights the phenotype due to parent-of-origin effect. On the contrary, in the social approach towards strangers mice, all the experimental groups, except for AKR/J animals showed curiosity and propensity to interact with unknown animals. AKR/J animals instead, showed less ability to socialize with unknown animals, maybe due to a more anxious behavior and, moreover, they seemed to not be able to distinguish between the two different animals. In novel object recognition test we didn't find differences between groups and, even if all of them showed to be able to distinguish between the known and the unknown objects, their long-term memory was poor.

Altogether, this work provides a wide characterization of the parent-of-origin effects on some complex traits, such as sleep, cognitive performance and neuronal activity in baseline condition and in response to sleep deprivation. Moreover, we demonstrated that perinatal environment and maternal cares

play a role in some behavioral expression, in particular the social ones, while they seem to not affect exploring behavior related to objects and on long term memory.

Limitation of the research

In this study we used reciprocal crosses of AKR/J and DBA/2J inbred strains to investigate role of parent-of-origin effects in complex phenotypes such as sleep architecture, homeostatic response to sleep deprivation and cognitive behavior. However, to better characterize our experimental groups and how perinatal environment and maternal cares can affect adult's behavior, we should have included in the study also the progenitors and the reciprocal crosses grown up with biological mother.

In addition, in our study we tried to investigate differences one the medial PFC activity between the two strains at the BL and after SD. Perhaps the neuronal activity in the mPFC should be also tested during behavioral test aimed at understanding how SD alters the neuronal activity in relation to a specific cognitive task.

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