

1 Activity of Amygdala in altruistic decision making

In this chapter, a new aspect of the behavioural analysis from in vivo tasks on mice will be investigated: the ability of mice to display empathic-like behaviours, such as **altruistic** choices.

Through the *altruism task*, two mice are put in a situation in which they can choose whether to perform an altruistic or selfish choice, while having their neural activity recorded. In this context, however, the employed technique for calcium imaging is not microendoscopic calcium imaging, but *Fiberphotometry* (discussed in 1.4), from which an overall signal containing the calcium activity is obtained.

As for the area of the brain under investigation, in this case the choice went back on the **basolateral amygdala (BLA)**, which has been shown to be connected with prosocial choices in rodents (1.1).

After describing the results of the altruism task in terms of behaviour, the focus will regard the analysis on the calcium tracks from fiberphotometry, and in particular their relative change with respect to a baseline activity, such as the effect that neuronal silencing provokes on empathic behaviours.

1.1 Social decision making in mammals

In order to live as a group, mammals have to perform decisions based on their *social interactions*. Often, such decisions are strictly egoistic and selfish, in order to survive as single individual and obtain the best for itself. However, evolution taught us that **altruistic** decisions, engaged with the purpose of creating a benefit for the entire community, are present as well, and are actually necessary to the surviving in social groups [Batson, C. D.].

The reasons behind whether a social choice may be of altruistic rather than selfish type are often non trivial and may depend by several factors, such as the presence of a *dominance* relation inside a group, the degree of closeness between two individuals, or biological factors like age and gender.

In the first years of study of social interactions and decision making, the term *altruism* was used only for humans, but, through years, the increase of discoveries of similar attributes in other animals changed the situation. For example, frequent habits of sharing food has been observed in parrots [Brucks, D. & von Bayern] and monkeys [Krupenye, C., Tan, J. & Hare]. Mice do not differ: rodents have been shown to display altruistic decision making such as consolatory and collaborative behaviours, such as offering help to conspecific in need [Bartal, I. B.-A., Decety, J. & Mason].

Moreover, a dysfunctional behaviour in social decision making, such as a lack of empathy, could be strictly related to many psyco-pathologic conditions such as Alzheimer's disease or dementia.

Through the years, the interest in social decision making observed in group of mammals has been growing. However, very often the focus of such studies stops at the *expressed* behaviour, rather than inspecting the neural causes behind it. Recently, studies on primates [Dal Monte, O., Chu, C. C. J., Fagan] seem to have identified the neural circuits responsible for social interactions and decision making in the basolateral amygdala (BLA), and following studies on mice

confirmed it as well [Felix-Ortiz, A. C., Burgos-Robles, A., Bhagat].

1.2 Description of the altruism task

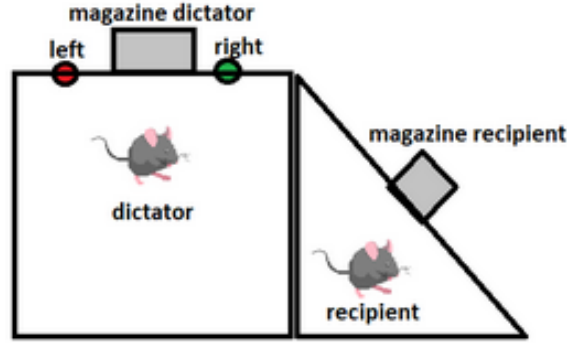


Figure 1: *Main setting of the altruism task: a dictator mouse (left) can choose between two pokes, one of which will deliver a food pellet to a recipient mouse (right) as well*

The *Altruism task* is a task in which two mice are tested in their social decision making. It consists in two mice located in adjacent compartments, separated by a metal mesh which allows them to see and sniff each other, but not enter in direct contact. One mouse, called the *recipient*, assumes only a passive role: it stays in its space and, when a food delivery happens from an apposite box (called *magazine*) it can eat the delivered food pellet. In contrast, the other mouse, called *dictator*, has to perform a social choice. Indeed, in any case a deliver of food to its own magazine will happen by poking one of two buttons available, but even if both types of pokes will provoke a pellet delivery for the dictator, only one of them will give food to the recipient as well. It follows that the two choices of the dictator can be classified in *selfish* and *altruistic* decisions. In order to stimulate the seeking of food, mice have been kept at 90% of their standard body weight before the test. The setting of the test has been the following:

- The tasks were performed considering both mice kept together in same sex pairs, for two weeks before the test
- The animals were tested for 5 days. The first day involves a *learning* process for the dictator to understand the food delivery mechanism. The main test is considered happening during the following 3 days, and finally the process is considered well assimilated by the dictator during the final day
- The position of the altruistic and selfish pokes (i.e. of the two buttons providing or not the food delivery to the recipient) has been randomized through the different days, in order to rule out spatial causes for the results

- A control test has been performed in parallel. Here, the recipient mice were replaced by inanimate objects, in order to determine the effective relevance of their presence in the task
- During the performance of the 5 days of test, the calcium activity in the basolateral amygdala of the dictator has been recorded using the Fiber-photometry technique, giving as result a collective signal (as discussed in 1.4)

1.3 Behavioural results in the altruism task

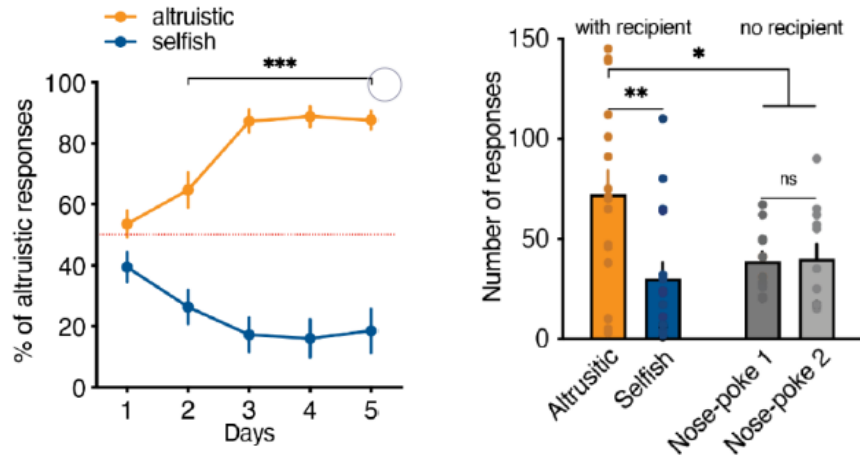


Figure 2: *Left: Percentage of change in altruistic response (i.e in the number of altruistic pokes) for altruistic and selfish mice. Right: difference in number of altruistic and selfish pokes by the dictator when the recipient is present or not*

The first evident result of the altruism task is that, when a recipient mouse was present and same sex pairs adopted, the dictator mice showed an increase in the number of altruistic pokes during the test days. In contrast, when recipient mice were replaced by inanimate objects, no appreciable difference between altruistic and selfish preferences have been detected. Such difference started to emerge from the second day of test, as confirm of the overcoming of a learning period in the first day.

An important difference has been observed between male and female mice: only male dictators showed altruism preference, while female ones did not show an appreciable difference between selfish and altruistic pokes.

Next, the spatial exploration of the mice has been under study. It came out that altruistic dictators (i.e. dictators which showed preference for altruistic pokes) tend to explore more the area in proximity to the recipient, in contrast with selfish dictators which didn't show particular interest in their partners.

To test whether the visual interaction is relevant to determine the altruistic preferences, the task has been repeated replacing the metal mesh with an opaque screen, which still allowed olfactory and auditory stimuli, but not visual ones.

In this situation, mice showed a marked decrease in the altruistic responses, with respect to the previous case in which metal mesh was used. This implies that the dictator mouse needs to be able to see the recipient partner getting the food delivery, in order to be able to decide for the altruistic alternative. Finally, to further determine the ability of dictator mice to *learn* from experience and change their habits in light of an altruistic choice, dictators were tested with no partner in adjacent cages, to trigger one nose poke over the other. Subsequently, the recipient mouse was put in the near cage, and the location of the altruistic poke, delivering food to it as well, has been assigned to the poke less pressed by the dictator. Despite this, the dictator showed through days a change in his preference towards the altruistic poke. Changing the recipient with an inanimate object, such preference decreased once again. The conclusion is straightforward: the presence of the recipient is the determining factor for the altruistic preference by the dictator.

Once, in normal conditions, the altruistic preference has been established, one may ask until which conditions such preference is maintained. At this purpose, the task was repeated changing the rules of food delivering. Now, two nose pokes were necessary for the food delivery in the altruistic poke, while only one in the selfish one. Even with this additional effort, males and females mice which previously showed altruistic preferences, increased the number of altruistic over selfish preferences also in this case. Increasing the necessary number of pokes to 4, male dictators kept their altruistic preferences, while female ones did not show anymore a significative difference. Finally, requiring 6 nose-pokes for altruistic delivery still kept male mice in their altruistic preference, while made female ones prefer the selfish one, and only from 8 pokes, also males stopped to be altruistic. Overall, these results showed that *male mice tend to share food with their cagemates partners, even at costly conditions*.

Finally, mice were tested in two more conditions: absence of rewards for both dictator and absence of reward for dictator, but not recipient mouse. While, in the first case, no preference between the two pokes were expressed by the dictator, in the second one there was an altruistic preference. This means that the dictator can choose an altruistic decision even if there is no direct benefit for itself.

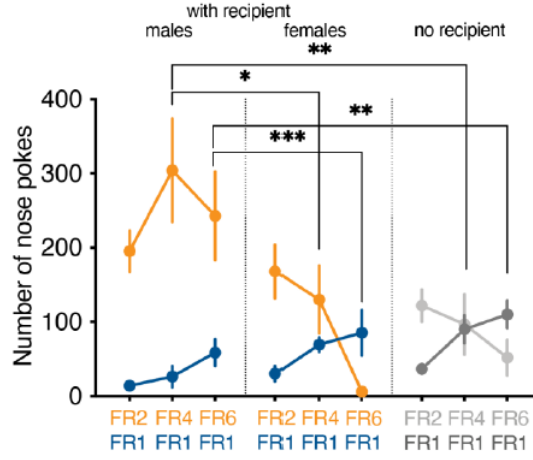


Figure 3: *Number of nose pokes of male and female mice, with and without recipients.*

1.4 The role of social hierarchy in altruism display

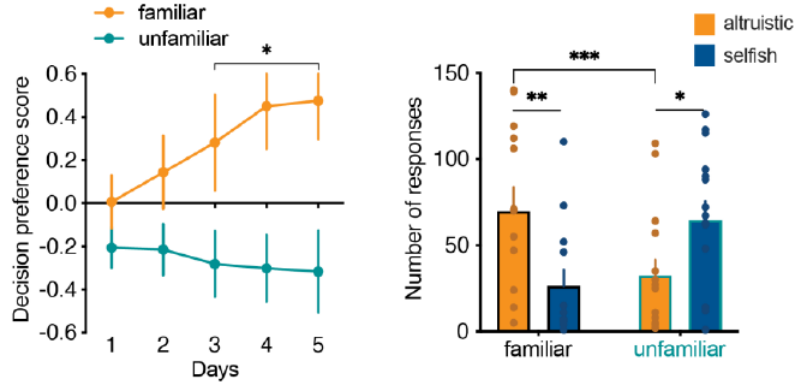


Figure 4: *Left: decision preference score with familiar and unfamiliar recipients. Right: Number of responses by selfish and altruistic dictators, with familiar and unfamiliar recipients*

By today, it is known that familiarity between individuals plays a key role in social relationships [De Waal, F. B. M. & Preston]. In the case of the altruism task, it is to be expected that the shown altruism by the dictator is strictly dependent on the period preceding the test, in which dictator and recipient have been cagemates for two weeks. To test this hypothesis, the task has been repeated using unfamiliar recipients, and this implied a strong abolishment of altruistic choices, where most of dictator mice actually showed preferences for the selfish pokes. This has been measured through the **decision preference**

score

$$DPS = \frac{A - S}{A + S} \quad (1)$$

Where A and S are the number of altruistic and selfish responses. Next, a *dominant/follower* relationship has been established through the **tube test**, in which two mice have been paired in a tube allowing the passage of just one individual. This test helped to figure in the pair who was the *dominant* individual, i.e. the one pushing the other, and the *follower* individual, i.e. the one retreating. The dominance was quantified thorough the **Normalized David's score** [De Vries, H., Stevens, J. M. G. & Vervaecke]

$$NDS = \frac{1}{N} \left(DS + \frac{N(N-1)}{2} \right) \quad (2)$$

Where N is the number of tested subjects and DS is the David's score, i.e. the ratio of wins.

Analyzing 9 pairs of mice used in the altruism task, the main results obtained were the followings:

1. The dominance relationship is *transitive*: if mouse A is dominant with respect to mouse B, and mouse B is dominant with respect to mouse C, then mouse A dominates mouse C as well
2. Dictator mice showing selfish preferences in the altruism task displayed low scores in the NDS compared to their recipients, which means they tend to be less dominant
3. No significant differences have been detected between the dominance scores of altruistic dictators and their recipients
4. The more a dictator has a higher dominance value NDS , the more he is willing to perform altruistic preferences in the altruism task

Next, the study tested the hypothesis that *an increase in altruism is related to an increase in affective state matching between individuals*. To do so, the recipient has been subjected to a manipulation of its emotional state, in this case a fear conditioning paradigm through little shocks. In the meantime, the dictator mouse (here assuming the role of *observer*) was allowed to see the recipient through a transparent partition.

The main result was that altruistic mice displayed an increased freezing behaviour during this phase, compared to selfish ones. And being these mice the dominant ones, this lead to the conclusion that *dominant mice tend to show more empathic like behaviours*, from altruism to emotional contagion.

1.5 The role of BLA neurons in empathism

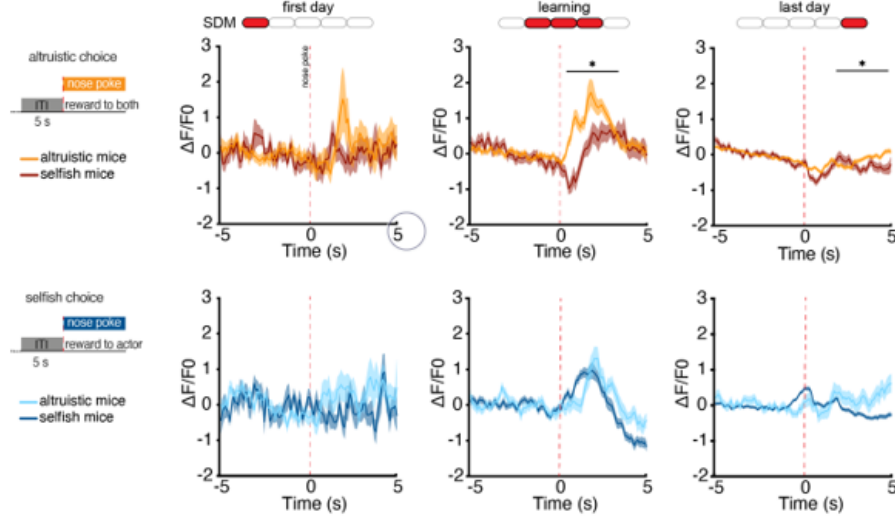


Figure 5: *Top: PSTH of selfish and altruistic mice during nose pokes in altruistic pokes. Bottom: PSTH of selfish and altruistic mice during nose pokes in selfish pokes. Results have been averaged on all nose pokes*

The fiberphotometry analysis of the calcium activity in the BLA allowed to establish a connection between the type of choice adopted by dictator mice and their corresponding neural activity. In order to analyze the relative change of such activities during altruistic or selfish pokes, the raw data from the photometry have been subjected to a **peristimulus time histogram (PSTH)** analysis. Such analysis goes through the following steps:

1. Set a **START** and **STOP** time for the analysis, i.e. set the time window in which considering the variation of the neural activity (in this case $START = STOP = 5s$ for a window of $10s$)
2. For every time point x_t of the overall activity given by Fiberphotometry, consider the interval $[x_t - START, x_t + STOP]$
3. Compute the mean μ_t and standard deviation σ_t of the activity in such interval
4. The value of the PSTH for the selected time point is then

$$P_t = \frac{x_t - \mu_t}{\sigma_t}$$

Next, in order to quantify the intensity of the activity, **areas under the curve (AUC)** were estimated numerically via trapezoid method [Quarteroni-Sacco].

The overall analysis on raw photometry data, PSTH tracks and AUC showed some remarkable results:

- Dictator mice showed an increase activity in the BLA when a recipient mouse was present, compared to when an inanimate object was placed in the near cage
- During the first day, in all cases no significant differences emerged in the activation of BLA, such as in the last day
- In the intermediate days of learning, however, altruistic mice showed a strong activation during the nose poking in the altruistic pokes, but not in the selfish ones. Selfish mice, in contrast, did not show any particular activation of their neural activity during both pokes.
- The AUC analysis suggested that the levels of activity were higher during the learning phase, rather than first and last day

To further inspect the role of BLA neurons in empathy display, such neurons have been silenced via a **chemogenetic procedure** [Qi-Gang Zhou, Ashley D Nemes]. In this approach, a viral vector, carrying specific receptors, is injected in the area of interest. Such receptors, called **hM4D receptors**, belong to the group of *inhibitory designer receptors exclusively activated by designer drugs (DREADD)*, which activate themselves only when in contact with a particular drug, the **Clozapine N-oxide (CNO)**. In this way, mice injected with hM4D-CNO will exhibit an *inhibition* of BLA-neurons. In contrast a control group of mice was injected with CNO only, without hM4D receptors.

The obtained results after these injections showed a significant reduction of freezing behaviour in the dictators, while their recipient partners were subjected to emotion conditioning as previously described. This confirms what previous studies [Allsop, S. A. et al] showed, namely a *critical role of the amygdala in emotional matching*. Silencing BLA neurons revealed to be correlated as well with the amount of altruism preferences. Indeed, mice with silenced BLA neurons failed to show a marked preference for altruistic choices, in contrast with the standard case. Indeed, while approximately 3 out of 4 mice showed altruistic preferences in standard conditions, a similar percentage of BLA-silenced mice showed preference for selfish pokes.

BLA silencing did not affect the number of responses or the latency of choices, rather it affected the type of the social decision. This is a further evidence of the primary role of BLA in altruism display.

Finally, the silencing of BLA neurons appeared to reduce the dominance as well: a higher number of BLA-silenced mice assumed a subordinate role with respect to control mice. In particular, if transitive relationships of dominance are distributed in levels $\alpha \rightarrow \beta \rightarrow \gamma \rightarrow \delta$, silenced mice belong only to levels γ and δ .

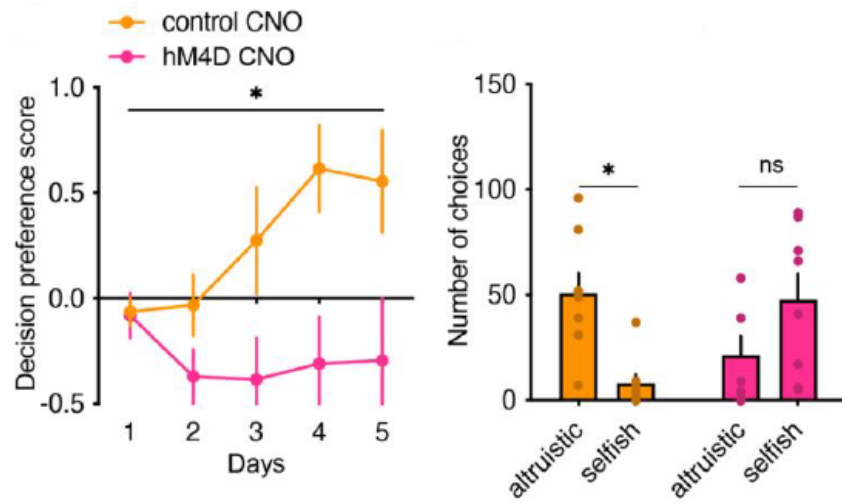


Figure 6: *Left: Decision preference score in BLA-silenced mice (hM4D CNO) and non silenced mice (control CNO). Right: number of selfish and altruistic pokes in BLA-silenced mice (hM4D CNO) and non silenced mice (control CNO)*

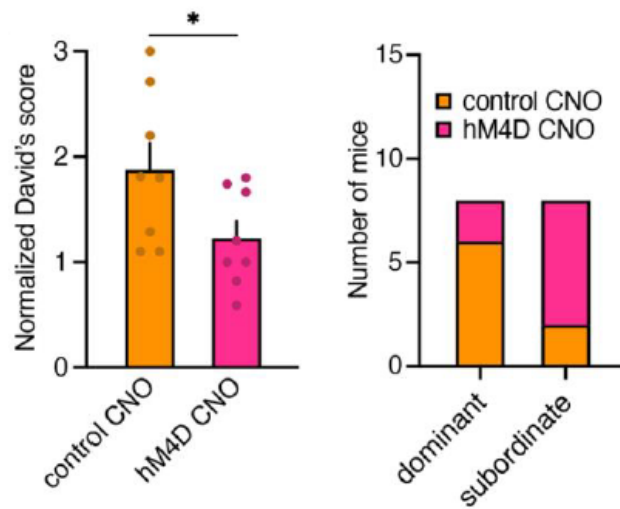


Figure 7: *Left: Normalized David's score in BLA-silenced mice (hM4D CNO) and non silenced mice (control CNO). Right: number of dominant and subordinate mice in BLA-silenced mice (hM4D CNO) and non silenced mice (control CNO)*